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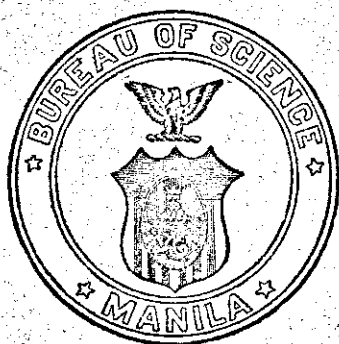
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Committee on Experimental Medicine

V. G. HEISER, M. D.; W. E. MUSGRAVE, M. D.; B. C. CROWELL, M. D.
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THE PHILIPPINE JOURNAL OF SCIENCE

B. TROPICAL MEDICINE

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EXPERIMENTAL BALANTIDIASIS

By ERNEST LINWOOD WALKER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Seven plates

Balantidiasis is the intestinal parasitization of man with the ciliated protozoan, *Balantidium coli* Malmsten, which may give rise to a chronic diarrhoea or a fatal dysentery. The first case reported was by Malmsten at Stockholm in 1857. Strong (1904) was able to collect from the literature 125 cases of balantidiasis. Since then and up to the present time 12 cases, of which the literature is available, have been reported. Therefore, in the fifty-six years following the discovery of the parasite only about 137 cases of infection of man have been reported in medical literature. This makes it appear that balantidiasis is a comparatively rare infection, and consequently of more scientific interest than practical importance.

In the Philippine Islands, however, parasitization with this protozoan appears to be relatively prevalent. The first case described here was by Strong in 1904. Subsequently a few cases were reported, notably 3 fatal cases with necropsy by Bowman (1909 and 1911). Willets (1913) found 2 cases in the examination of 400 stools in the Batanes Islands, north of Luzon, and I found 2 cases in the examination of 48 stools at San José, Mindoro. Thirteen cases have been observed at the Philippine General Hospital. In the Bilibid Prison 35 cases have been found in the last two and a half years, an average of more than 1 a month. From March 4 to March 25 of the present year, a period of twenty-one days, 8 new cases of parasitization with

this protozoan were discovered. Moreover, on account of the infrequent appearance of the parasites in the stools of infected persons and the absence of clinical symptoms in many of the cases, it is probable that parasitization with *Balantidium coli* is frequently overlooked in the routine examination of stools.

The geographical distribution of this infection is wide, and appears to be influenced by certain sanitary conditions rather than by climate. Cases have been reported in Russia, Sweden, Finland, Germany, Italy, North America, South America, Cuba, Africa, Sunda Isles, Cochin China, and the Philippine Islands. It is noteworthy that most of the known cases have occurred in Russia and Scandinavia, cold countries, on the one hand, and, on the other hand, in a tropical country, the Philippine Islands.

Balantidiasis in man is characterized clinically by the appearance of *Balantidium coli* in the stools and, in a certain proportion of the cases, by a diarrhoea or dysentery.

However, the appearance of the parasites in the patient's stools is very irregular; they may be absent for weeks at a time, and they may at any time be found only in very small numbers and, consequently, frequently are discovered only when attention is attracted to the stools by the appearance of a persistent diarrhoea or of dysenteric symptoms.

The dysenteric symptoms are likewise an extremely inconstant manifestation of the infection. Of the 132 human cases of which I have been able to obtain access to the literature, 130 had diarrhoeal or dysenteric symptoms; but it is probable that the majority of these cases were brought to the attention of the physician by the dysenteric symptoms and that the parasites were found only in the attempts to discover the etiology of the symptoms. Of the 57 cases of parasitization so far reported in the Philippine Islands, only 11 have shown diarrhoeal or dysenteric symptoms. In other words, many of the infections are latent.

The mortality in 111 cases according to Strong (1904) was 32, or 29 per cent. The primary cause of death in some of these cases may have been other than the balantidiasis; on the other hand, many of the patients passed from observation and their ultimate fate was unknown. It is probable that if the patients parasitized, but not infected with the parasite, be included the percentage of mortality would be considerably lower, while if only those patients in which there was an actual invasion of the tissues by the balantidia be considered, the death rate from balantidal infection would be materially increased. The mortality in 57

recent cases in the Philippines has been up to the present time 4, or 7 per cent, but most of these are latent cases without symptoms.

The pathological changes in balantidiasis, as reported in the literature of 40 necropsies, showed ulcerations of the large intestine in 36, chronic catarrh in 3, and in 1 case the condition of the large intestine was not given. The ulcers are said not to be characteristic. The old ulcers are described as blackish or slate-colored and the recent ulcers are frequently slightly undermined and irregular. The mucosa between the ulcers is usually reddened and hæmorrhagic. In only 1 case (Peterson, 1873) has an ulcer been reported in the ileum. The parasites were found in the contents of the large intestine at necropsy in 21 of the cases. In all of these cases the ulcerative lesions were probably complicated by secondary bacterial invasion.

Histological studies of the lesions in balantidiasis have been made in a few of the fatal cases, notably by Strong and Musgrave (1901), Solowjew (1901), Bowman (1909 and 1911), and Bel and Couret (1910). The principal changes recorded are catarrh of the mucosa, cell degeneration and necrosis, polymorphonuclear infiltration (probably due to secondary invasion of the lesions by bacteria), hypertrophy of the vessels, hæmorrhages, round cell infiltration, eosinophilia, and penetration of the parasites into the sound tissues underlying the ulcers. The balantidia were found in the submucosa, muscularis, in the mesocolic lymph glands, and in the blood vessels and lymph spaces, lying singly or in groups.

Unlike infections with *Entamæba histolytica*, liver abscess does not appear to be a common complication in balantidiasis; but Stockvis (1884) reported a case in which the balantidia were found in the sputum of the patient and were believed to have come from a liver abscess which had ruptured into the lung. The patient recovered, therefore no necropsy was obtained.

Besides man, monkeys and pigs are known to be naturally parasitized with balantidia.

Brooks (1902) reported an epizootic of dysentery among the apes in the New York Zoölogical Park due to balantidial infection. Noc (1908) found a case of balantidial dysentery in *Macacus cynomolgus* at the Pasteur Institute in Saigon, Indo China. Brumpt (1909) reports 6 cases of natural parasitization of *Macacus cynomolgus* with balantidia in Indo China.

Balantidia were first observed as parasites of the domesticated pig by Leuckart (1861 to 1863). They have been found by

Stein (1862), Ekecrantz (1869), and Wising (1871) in the pigs in Sweden, by Grassi (1882) in Italy, by Rapchevski (1882) in Russia, by Railliet (1886) and by Newmann (1888) in France, by Stiles (Strong, 1904) in the United States, and by Strong (1904) in Manila. The examination of the fæces of a somewhat limited number of pigs here convinces me that a large proportion of them in the Philippine Islands are parasitized with this protozoan. The balantidium found in the intestine of the pig is generally considered not to produce any symptoms or lesions in its host.

The question of the identity or nonidentity of the balantidium found parasitic in the pig with *Balantidium coli* of man has an exceedingly important bearing on the endemiology and prophylaxis of balantidiasis.

Wising (1885) and Grassi and Calandruccio (1888) were of the opinion that the balantidium of the pig is a species distinct from *Balantidium coli* of man. Wising states that the latter species is smaller and does not become encysted, while the pig balantidium is larger and usually appears in the fæces in the encysted stage. Grassi and Calandruccio drew their conclusions from the fact that they were unable to infect themselves with the balantidium of the pig by the ingestion of the encysted parasites. On the other hand, recent authors are inclined to the opinion that the balantidium of the pig and of man are one and the same species, but no special evidence is advanced to support this view.

In consideration of the close domestic relations existing between the pig and natives of the lower class, the fact that the pig is the chief scavenger in these Islands, the prevalence of balantidium as a parasite of the pig, and the fact that this parasite is passed more or less constantly in the fæces of the parasitized pig in the resistant encysted stage, make the identity of the 2 species particularly important in explaining the prevalence of infections of man with *Balantidium coli* in the Philippine Islands.

The experimental infection of animals with *Balantidium coli* has been attempted by many investigators. Brumpt (1909), who found cases of natural infection of apes with *Balantidium coli* in Indo China, reports that he was able to pass one of these strains through 6 healthy monkeys, to parasitize a monkey with balantidia obtained from the fæces of a pig, and to parasitize pigs with the strain of balantidium from the monkey. The incubation period of the parasite varied from two to seventeen days. A diarrhoea developed in 1 of the parasitized monkeys

and 1 of the pigs, and the stools of the pig contained blood and balantidia filled with red blood corpuscles. At the necropsy performed on the parasitized monkey, balantidia were found in the large intestine from the cæcum to the anus, but there were no lesions. In the necropsy of the infected pig the author states that lesions were present in the large intestine which were identical with those described by Strong, Askanazy, and others in human cases. No histological examinations appear to have been made in any of Brumpt's experimental infections to determine the etiologic relation of the balantidia to the lesions.

On the other hand Ekecrantz (1869), Wising (1871, 1885), Rapchevski (1880), Lavrovskaya (1890), Afanasyeff (1891), Casagrandi and Barbagallo (1896), Chigayeff (1898), Valayeff (1898), Zhegaloff (1899), Chichulin (1900), Strong (1904), and Bowman (1911) have failed in their attempts to infect animals (cats, rabbits, dogs, pigs, monkeys) with *Balantidium coli*. Casagrandi and Barbagallo state, as a conclusion from numerous experiments, that the parasites could sometimes live in the intestine of cats if a catarrhal condition was first produced, but that they were incapable of producing independent disease in the intestine. Bowman injected fresh fæces from a case of severe infection in man many times into the rectum of monkeys suspended by the lower extremities in order that none of the infective material could be evacuated. He performed a colotomy on another monkey and injected 20 cubic centimeters of a balantidial stool on two occasions into the colon. And, finally, tissue from an ulcer containing balantidia was inserted beneath the mucosa of the colon of a monkey and sutured in place. In every case the results were negative; the parasites were never found in the fæces of the experimental animals.

The following infection experiments on monkeys were undertaken to discover the reason for the discrepancy in the results of Brumpt and of other authors in their attempts to infect animals with *Balantidium coli*, to determine the identity or non-identity of *Balantidium coli suis*¹ with *Balantidium coli hominis*, and to obtain further information on certain obscure points in the etiology, pathology, and endemiology of balantidiasis of man.

The monkeys used in these experiments were healthy individuals of the common species found on the Island of Luzon, name undetermined. Some of them had been in captivity for some

¹ *Suis* or *hominis* is attached to the specific name throughout this paper for convenience in distinguishing the pig and the human strains and not for the purpose of indicating taxonomic varieties of *Balantidium coli*.

time, being kept in rooms in the animal house; others were freshly purchased by this Bureau and were presumably recently captured. No spontaneous infections with *Balantidium coli* have ever been discovered in the large number of monkeys that have been used in the biological laboratory. Owing to the fact that in infections of monkeys with this protozoan the parasites rarely appear in the stools, it was considered useless to attempt to control their freedom from natural parasitization by microscopic examination of their stools. The monkeys were kept in individual cages throughout the course of the experiments.

These monkeys were either fed or injected rectally with the faeces containing the balantidia, depending upon the stage of development of the parasites. If the balantidia were encysted, as was more frequently the case when they came from the pig, a portion of the faeces containing the cysts was mixed with the food of the monkey. On the other hand, motile balantidia, which are more frequently found in human stools, were injected rectally, since it is believed that the parasites in this unprotected stage are incapable of withstanding passage through the stomach. The diarrhoeal stool, diluted if necessary with physiological salt solution, was given as a high rectal injection through a rectal tube attached to a large syringe. Repeated feedings were given in many cases in order to insure parasitization of the experimental animal, which could not be readily determined by stool examinations on account of the infrequent appearance of the parasites in the faeces, and because the monkeys sometimes refused to eat the infective food. That repeated feedings are not necessary, however, to secure parasitization of monkeys with balantidia is proved by the fact that several animals which were fed or injected rectally only once became parasitized.

Balantidium coli suis was used in more of these experiments than *Balantidium coli hominis* because human cases that showed a sufficient number of the parasites in their stools were not frequently obtainable, and, moreover, it was especially desired to determine the parasitism and pathogenesis of the balantidium of the pig for the monkey.

Following the feedings or rectal injections, the stools of the monkeys were saved daily, or at frequent intervals, and examined macroscopically for diarrhoea or dysentery and microscopically for the presence of balantidia.

At the beginning of the investigation it was planned to kill the experimental animals at regular intervals in order to study the progress of the infection, but this intention was interfered

TABLE I.—The experimental parasitization of monkeys with *Balantidium coli suis* and *Balantidium coli hominis*.

Monkey No.	Date of feeding or injecting.	Method of infection.	Source of material.	Stage of development of balantidia.	Stool examination.		Clinical manifestations.	Fate of monkey.	Days under observation.	Necropsy.	Histological examination.	Parasitization.	Infection.
					Result.	Date.							
10	Sept. 11 and 13, Oct. 7 and 8, 1912	Fed.	Pig	Encysted	Balantidia	Oct. 11, 1912	Diarrhoea with flakes of bloody mucus; very sick, Feb. 9, 1913.	Killed, Feb. 10, 1913	152	Colitis and ulceration of large intestine; no balantidia.	Superficial ulceration; hyperemia; polymorphonuclear infiltration; no balantidia.	+	0
11	Oct. 14, 1912	do	do	do	do	Nov. 4, 1912	Negative	Killed, Nov. 21, 1912	38	Slight colitis; balantidia	Hyperemia; punctiform hæmorrhages; eosinophile infiltration of mucosa; balantidia in mucosa and blood vessels of submucosa.	+	+
26	Dec. 21, 23, 24, 27, 28, and 30, 1912	do	do	do	do	Jan. 6, 1913	do	Died, Apr. 14, 1913	114	Colitis; no balantidia	Catarrhal exudate; no balantidia	+	0
27	do	do	do	do	do	Jan. 4, 1913	do	Died, Feb. 3, 1913	44	Colitis; balantidia	Catarrhal exudate; minute superficial ulcers; polymorphonuclear infiltration; no balantidia.	+	0
28	do	do	do	do	do	Dec. 27, 1912	do	Killed, Mar. 19, 1913	88	do	Catarrhal exudate; no balantidia	+	0
29	do	do	do	do	do	Jan. 10, 1913	do	Died, Feb. 17, 1913	58	Negative	None made	+	0
30	do	do	do	do	do	Jan. 21, 1913	do	Killed, Mar. 4, 1913	73	do	do	+	0
31	Feb. 21, 1913	Rectal injection	Man	Motile and encysted	do	Mar. 8, 1913	do	Died, Apr. 4, 1913	42	Large intestine normal; many balantidia	Balantidia in mesenteric lymph glands; no cellular reactions.	+	0
32	Feb. 21 and 22, 1913	Fed.	do	Encysted	Negative	do	do	Died, Apr. 16, 1913	54	Negative	None made	0	0
33	Mar. 7, 1913	Rectal injection	do	Motile	do	do	do	Died, Mar. 31, 1913	24	do	do	0	0
34	May 26, 1913	do	do	do	do	do	do	Died, June 7, 1913	12	Colitis; no balantidia	Diphtheritic exudate; slight polymorphonuclear infiltration; no balantidia.	0	0
35	do	do	do	do	Balantidia	June 1, 1913	Bloody mucous stools on day of death.	Died, June 8, 1913	10	Colitis; balantidia	Hæmorrhagic exudate; some destruction of epithelium; no balantidia.	+	0
36	June 6, 1913	do	Pig	do	do	June 18, 1913	Negative	Killed, July 26, 1913	50	Negative	None made	+	0
37	do	do	do	do	Negative	do	do	do	50	do	do	0	0
38	June 6, 11, 12, 13, and 14, 1913	Fed.	do	Encysted	Balantidia	July 2, 1913	do	do	50	do	Negative	+	0
39	do	do	do	do	do	June 26, 1913	do	do	50	Slight colitis; no balantidia	Slight hæmorrhagic exudate; eosinophile infiltration; no balantidia.	+	0
40	June 20, 21, 23, and 25, 1913	do	do	do	do	July 22, 1913	do	Killed, July 28, 1913	38	Slight colitis; balantidia	Slight diphtheritic exudate; punctiform hæmorrhages; eosinophile infiltration of mucosa.	+	0
41	do	do	do	do	Negative	do	do	do	28	Negative	None made	0	0
42	do	do	do	do	Balantidia	June 22, 1913	do	do	23	Slight colitis; no balantidia	do	+	0
43	do	do	do	do	do	June 28, 1913	Diarrhoea, very sick, July 25, 1913.	Killed, July 25, 1913	25	do	Congestion of mucosa; eosinophile infiltration; no balantidia.	+	0
44	June 24, 1913	Rectal injection	do	Motile	Negative	do	Negative	Killed, July 28, 1913	34	Negative	None made	0	0
45	do	do	do	do	do	do	do	do	34	do	do	0	0

with by the fact that many of the parasitized monkeys did not become infected and by the death of a number of the monkeys during the course of the experiments. The deaths of these monkeys were probably due in most cases to the close confinement in small cages and to improper food. In consequence of these unforeseen complications and in consideration of the fact that the symptoms and pathology of the later stages of infection with *Balantidium coli* have been adequately studied in human cases, it was decided to confine the investigation to the parasitization and early stages of infection which are in need of elucidation.

Post-mortem examination, with special reference to lesions in the large intestine and to the presence of balantidia in the intestinal contents, was made of each monkey that died or that was killed after feeding or rectal injection with balantidia. In every case where a colitis or ulcerations of the large intestine were present, pieces of the tissues were fixed and subjected to a histological examination for cellular changes and for the presence of balantidia.

These experiments are summarized in Table I.

Of the 13 monkeys fed encysted balantidia from the pig, 12 became parasitized; and of the 4 monkeys that received rectal injections of motile balantidia from the pig, none became parasitized. Only 1 monkey was fed encysted balantidia from man and he did not become parasitized. Four monkeys received rectal injections of motile balantidia from man, and of these 2 became parasitized. Therefore 12, or 70.6 per cent, of the monkeys fed or injected with *Balantidium coli suis*; and 2, or 40 per cent, of the monkeys fed or injected with *Balantidium coli hominis*, became parasitized. The smaller percentage of monkeys parasitized with the balantidia from man may be due to several causes: to the smaller number of experiments with material from this source, that the infective material contained fewer balantidia, and to the fact that most of the experiments with the human strains were with motile forms administered by rectal injection.

In pigs parasitized with balantidium the parasites appear rather constantly in the encysted stage in the formed stools, and often in enormous numbers. On the other hand, in parasitized man the balantidia are rarely found in the encysted stage in formed stools, but usually only in the motile stage in diarrhoeal stools, and then often at irregular intervals. In monkeys, Table II shows the number of stool examinations made on different days of each animal that became parasitized and the number of positive and negative findings for balantidia.

TABLE II.—Showing the number of times *Balantidium coli* was found in the stool examinations of parasitized monkeys.

Monkey No.—	Days under observation.	Total number of stool examinations.	Positive.	Negative.	Remarks.
10	152	44	2	42	Balantidia found at necropsy.
11	38	3	1	2	
26	114	53	12	46	
27	44	16	3	13	
28	88	55	1	54	
29	53	54	17	37	
30	73	39	3	36	
31	42	16	4	12	
32	54	22	0	22	
35	13	5	1	4	
36	50	33	2	31	
38	50	35	7	28	
39	50	31	13	18	
40	38	26	3	23	
42	38	28	1	27	Balantidia found on day after feeding.
43	35	23	5	18	

The small number of stool examinations made of some of the monkeys was due to my absence from Manila. With but few exceptions, the stools were formed and contained only encysted balantidia; when the stools were soft or diarrhœal, the balantidia were sometimes found in large numbers in the motile stage. In most of the positive examinations the balantidia were few in number, often only one or several to a cover slip. It would appear from these results that monkeys parasitized with balantidia from either the pig or man show a condition, with reference to the appearance of the parasites in the stools, more closely resembling man than the pig; that is, the balantidia appear rarely and in small numbers in the formed stools of the parasitized animal. In consequence of this the incubation period of the parasite, that is, the time elapsing between feeding or injecting the infectious material and the appearance of the balantidia in the stools of the parasitized animal, is of little significance.

These experiments, therefore, prove that monkeys are readily parasitized with either *Balantidium coli hominis* or *Balantidium coli suis*. Furthermore, they make it evident that the apparent failure of every previous investigator, with one exception (Brumpt, 1909), to parasitize animals—at least monkeys—probably has been due to the infrequency, and often total failure,

of the balantidia to appear in the stools of the experimental animal. And they demonstrate that *Balantidium coli suis* behaves, with reference to its appearance in the stools of the parasitized monkey, as does *Balantidium coli hominis*.

Monkeys 10, 35, and 43, two of which had and 1 of which had not become parasitized with the balantidia, had a diarrhoea or slight dysentery just before death; but, as will be seen from the post-mortem examinations, the dysentery was probably due to other causes than the balantidium infection. None of the other monkeys had diarrhoea or dysentery during the time they were under observation. However, as will be seen from the histological examinations of infected monkeys, the ulcerative process of this infection is probably extremely chronic, and consequently no dysenteric symptoms would be expected in these early stages of infection.

Post mortem, a number of the monkeys, whether killed or dying naturally, showed a colitis and sometimes ulcerations, which histological examination showed not to be due to balantidium infection. These lesions, at first misleading, were soon cleared up by the histological study of the tissues, and have served the useful purpose of comparison with the lesions produced by *Balantidium coli*, and for determining the part played by lesions of other etiology in the entrance of the balantidia into the tissues of its host.

A comparison of the gross lesions in the intestine of monkeys having an early balantidial infection with those having a colitis due to other causes has shown that the balantidial infection is characterized chiefly by the inconspicuousness of the lesions, which consist simply of reddened areas of the mucosa with or without punctiform hæmorrhages, sometimes so slight as to be overlooked, but no exudate or ulcerations; while the colitis of other etiology usually presents a catarrhal, diphtheritic, or hæmorrhagic exudate, frequently associated with ulcerations.

One, or 50 per cent, of the 2 monkeys parasitized with *Balantidium coli hominis* showed balantidia in the tissues post mortem. The negative animal had been parasitized only thirteen days when it died, while the one which showed balantidia in the tissues had been parasitized forty-two days when the post-mortem examination was made. Of the 13 monkeys parasitized with *Balantidium coli suis*, 1, or 7.7 per cent, showed balantidia in the tissues post mortem. This is a smaller per cent of infections than with the balantidium from man, but it is to be borne in mind that the series of animals parasitized with the latter

variety is very small from which to draw conclusions. The time of parasitization before necropsy of the monkeys that did not become infected with the balantidium from the pig varied from thirty-four to one hundred fifty-two days, and in the infected monkey it was thirty-eight days.

These experiments, therefore, prove that both *Balantidium coli hominis* and *Balantidium coli suis* are capable of invading the tissues and becoming tissue parasites of the monkey. They further show that only a small proportion of the monkeys parasitized with balantidia from either source become infected; that is, show the parasites in the tissues within the period of time (from thirteen to one hundred fifty-eight days) during which these animals were under observation. However, it is probable, since in these early infections little or no gross lesions are apparent, because only a few balantidia may have entered the tissues, and since the sections made can include only an infinitesimal part of the whole intestine, that more of these parasitized monkeys were really infected. It is also to be borne in mind that every one of the animals parasitized would be liable sooner or later to become infected. The condition is exactly similar to that found to prevail in entamoebic dysentery, in which only about 22 per cent of men experimentally parasitized became infected and developed dysentery (Walker and Sellards, 1913).

Histological examination of sections of the large intestine of the monkeys parasitized with *Balantidium coli* and showing a colitis has disclosed well-marked differences between those which did and those which did not contain balantidia in the tissues. The latter show either a catarrhal or diphtheritic exudate or ulcerations, usually associated with polymorphonuclear leucocyte infiltration of the mucosa and submucosa. On the other hand, sections of the intestine of cases of these early stages of infection with balantidia show the epithelium intact, except for mechanical injury due to entrance of the balantidia or to minute hæmorrhages, but no exudate or ulcerations. There is more or less congestion of the blood vessels and the tissue infiltration, which is slight, is of round cells and eosinophiles.

Therefore, the lesions and cellular reactions in balantidial colitis, in the early stage before complicated by secondary bacterial invasion, are characteristic and are distinguishable from those of colitis due to bacterial infection. Moreover, these cases have demonstrated that lesions in the intestinal epithelium from bacterial infection or other causes not only are not necessary for

the entrance of *Balantidium coli* into the tissues, but that in no one of the relatively large number of parasitized monkeys in which such lesions existed have the balantidia taken advantage of them to enter the tissues.

In their entrance into the tissues of the intestine the balantidia pass through the healthy epithelium. No necrosis or ulcerations of the epithelium are apparent, only a pushing aside of the cells or, at most, a mechanical rupture of the epithelium exists. In every case the entrance was effected through the epithelium between, and in no case within, the tubules. In monkey 10, parasitized for thirty-eight days with *Balantidium coli suis*, some of the balantidia are just penetrating the epithelium and others are in the mucosa between the tubules and, to a less extent, in the submucosa and in the blood vessels of the submucosa (Plates I, II, and III). In this case they were not found in the muscularis. In monkey 31, parasitized for forty-two days and, according to my assistant who performed the necropsy, showing no intestinal lesions, the balantidia were found in the muscularis and in the mesenteric lymph glands (Plates IV and V).

Therefore, these experimental infections have shown that both *Balantidium coli hominis* and *Balantidium coli suis* are capable of penetrating the sound intestinal epithelium and of wandering widely in the sound tissues of the mucosa, submucosa, muscularis, blood vessels, and mesenteric lymph glands.

In these early infections the balantidia are found singly or in scattered groups, evidently in the process of migration rather than of active multiplication in the tissues. In consequence, there is as yet little injury to the tissues beyond some mechanical rupture; and usually even this is wanting; the parasites, which are capable of amœboid movements, pass between the cells like migrating leucocytes. Moreover, there is in these early cases little cellular reaction in the vicinity of the single parasites and no extensive cellular infiltration of the tissues. At a later period an active multiplication of the balantidia takes place in the tissues, forming nests or colonies (Plates VI and VII) which, by their multiplication, aided probably by the secretion of a ferment, produce cellular reactions, necrosis of the tissues, and finally open ulcerations, which are advanced by the secondary invasion of intestinal bacteria.

That *Balantidium coli* is able, without the aid of bacteria, to produce abscesses and ulcerations of the intestine of infected

man, I am able to demonstrate with some sections of a human case of balantidiasis. The intestine from which these sections were obtained is from the necropsy of one of Bowman's (1909-1911) cases. In certain sections of this material, which is heavily infected with balantidia, I have been so fortunate as to find, in addition to open ulcerations, closed balantidial abscesses situated in the thickened submucosa, which lie under the sound mucosa and entirely surrounded by sound tissues, and which are, consequently, probably free from intestinal bacteria. An early stage of such an abscess is shown in Plate VI, consisting of a small cavity filled with balantidia and the infiltration of the surrounding tissues with mononuclear cells. Plate VII shows a part of an advanced abscess, which is too large to be shown in one field of even the low power of the microscope. Examined with high magnification the "pus" of this abscess is seen to consist, not of polymorphonuclear leucocytes, but of cell detritus and mononuclear cells only. The tissues about the abscess show round-cell infiltration, but no polymorphonuclear leucocytes. The absence of polymorphonuclear leucocytes in the "pus" and in the tissues surrounding the abscess confirms the opinion that the abscess is free from bacterial infection. Both the abscess and the sound tissues surrounding it contain many *Balantidium coli*.

Therefore, it having been demonstrated that balantidia are capable of penetrating the sound intestinal epithelium, that they do not invade secondarily the lesions due to bacteria, and that in the submucosa the balantidia are able to produce abscesses which later extend through the mucosa and become open ulcers, it would appear that the primary etiologic relation of *Balantidium coli* to balantidial dysentery had been proved.

SUMMARY AND CONCLUSIONS

1. Parasitization of man with *Balantidium coli* is relatively common in the Philippine Islands.
2. The balantidia appear in the stools of parasitized individuals only at irregular intervals, and consequently infections, unless accompanied by clinical symptoms, may frequently be overlooked.
3. A large proportion of the pigs in and about Manila are parasitized with balantidia.
4. Balantidia are passed in the resistant encysted stage more or less constantly in the stools of parasitized pigs.
5. Morphologically *Balantidium coli suis* is identical with *Balantidium coli hominis*.

6. Forty per cent of 5 monkeys fed or injected rectally with *Balantidium coli hominis* became parasitized.

7. Seventy and five-tenths per cent of 17 monkeys fed or injected rectally with *Balantidium coli suis* became parasitized.

8. Monkeys parasitized with either *Balantidium coli hominis* or *Balantidium coli suis* show the parasites in the stools only at infrequent intervals.

9. Only a small proportion of the parasitized monkeys became infected. Of 2 monkeys parasitized with *Balantidium coli hominis*, 1, and of 12 monkeys parasitized with *Balantidium coli suis*, 1, showed the parasites in the tissues post mortem.

10. The early lesions of the intestine of monkeys infected with *Balantidium coli* consist only of a slight hyperæmia with or without punctiform hæmorrhages.

11. Histological examination of the tissues of monkeys recently infected with *Balantidium coli* show changes, notably vascular dilation, minute hæmorrhages, round-cell infiltration and eosinophilia, which distinguish them from lesions of bacterial origin.

12. *Balantidium coli* was never found entering the tissues through the lesions in 10 parasitized monkeys having a colitis or ulcerations due to bacteria or other causes.

13. In those monkeys in which infection took place, the balantidia entered the tissues through the sound intestinal epithelium.

14. *Balantidium coli* can produce bacteriologically-sterile abscesses in the submucosa of an infected intestine.

15. *Balantidium coli* is the primary etiologic factor in the symptoms and lesions of balantidial dysentery.

16. The latency prevalent in balantidiasis of man is due chiefly to the fact that the patient, although parasitized, is not infected with *Balantidium coli*, but in part to the chronicity of the ulcerative process in infected cases.

17. Every person parasitized with *Balantidium coli* is liable sooner or later to develop balantidial dysentery.

18. *Balantidium coli suis* is identical with *Balantidium coli hominis*.

19. The domesticated pig is the chief source of infection in the balantidiasis prevalent in the Philippine Islands.

20. Therefore, efficient prophylactic measures against balantidiasis in the Philippine Islands should be directed against these animals, which should be confined and not allowed to run in yards and dwellings.

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ILLUSTRATIONS

(From photomicrographs by Charles Martin)

- PLATE I. Section of the large intestine of monkey 11. A single *Balantidium coli suis* under the healthy intestinal epithelium. Note the mechanical rupture of the epithelium which the parasite has apparently caused in entering the tissues, the absence of polymorphonuclear leucocytes, and to the extreme left the punctiform hæmorrhage with exuding red blood corpuscles.
- II. Section of the large intestine of monkey 11. Three *Balantidium coli suis* in the deeper part of the mucosa. Note that the balantidia are in the tissues between, and not within, the tubules and the nature of the cellular reactions.
- III. Section of the large intestine of monkey 11. A single *Balantidium coli suis* in a blood vessel of the submucosa.
- IV. Section of a mesenteric lymph gland of monkey 31. Several *Balantidium coli hominis* in the edge of the glandular tissue.
- V. Section of mesenteric lymph gland of monkey 31. A single *Balantidium coli hominis* in the center of the gland, only a part of which is shown in the figure. Note the cross section of a blood vessel adjacent to the balantidium.
- VI. Section of the large intestine of a man dead from balantidial dysentery. An early stage of a balantidial abscess in the submucosa. Note the small cavity filled with balantidia and the infiltration of the surrounding tissues with mononuclear cells.
- VII. Section of the large intestine of a man dead from balantidial dysentery. A part of an advanced balantidial abscess in the submucosa. The abscess is entirely surrounded by sound tissues. Note the abscess cavity and necrotic material and the balantidia and mononuclear cell infiltration in the surrounding tissues.



PLATE I. SECTION OF LARGE INTESTINE OF MONKEY 11, SHOWING A SINGLE BALANTIDIUM COLI SUI UNDER THE HEALTHY MUCOSA.



PLATE II. SECTION OF LARGE INTESTINE OF MONKEY 11, SHOWING THREE BALANTIDIUM COLI SUI IN THE DEEPER PART OF THE MUCOSA.

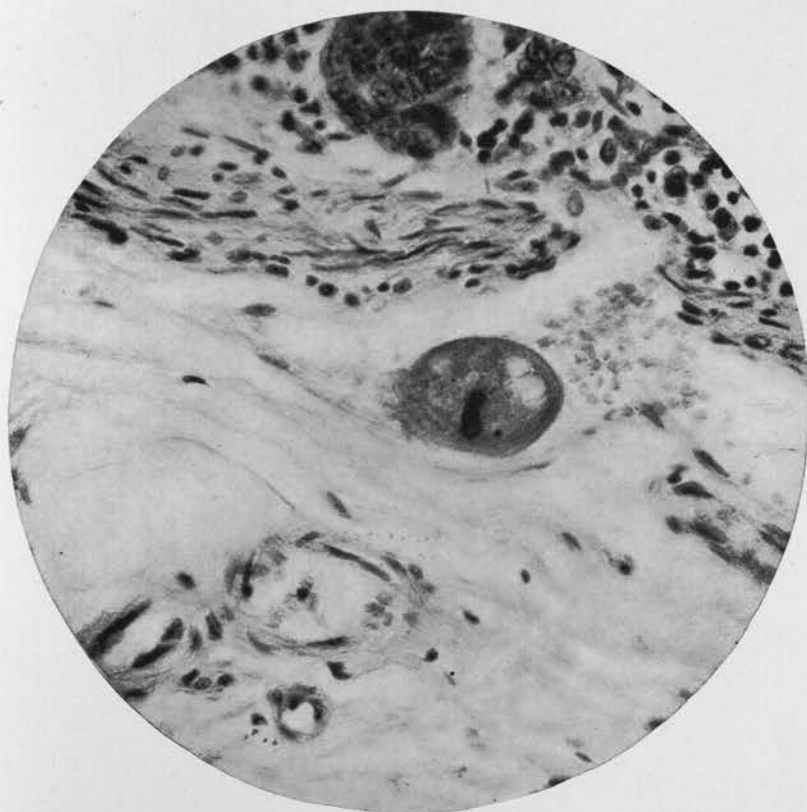


PLATE III. SECTION OF LARGE INTESTINE OF MONKEY 11, SHOWING A SINGLE BALANTIDIUM COLI SUI IN A BLOOD VESSEL OF THE SUBMUCOSA.

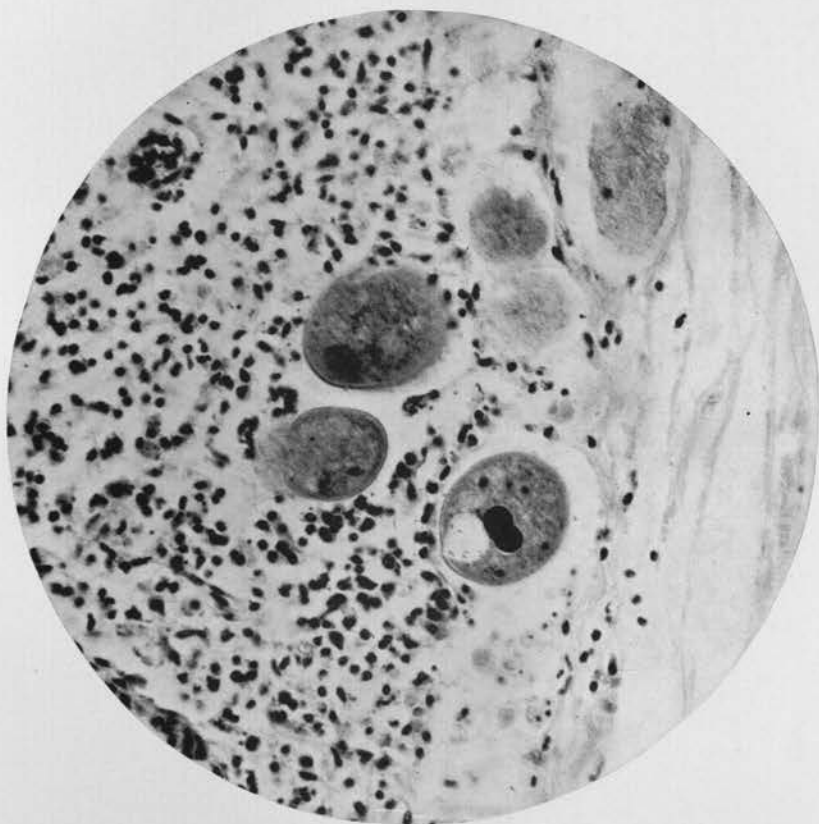


PLATE IV. SECTION OF A MESENTERIC LYMPH GLAND OF MONKEY 31, SHOWING SEVERAL BALANTIDIUM COLI HOMINIS IN THE EDGE OF THE GLANDULAR TISSUE.

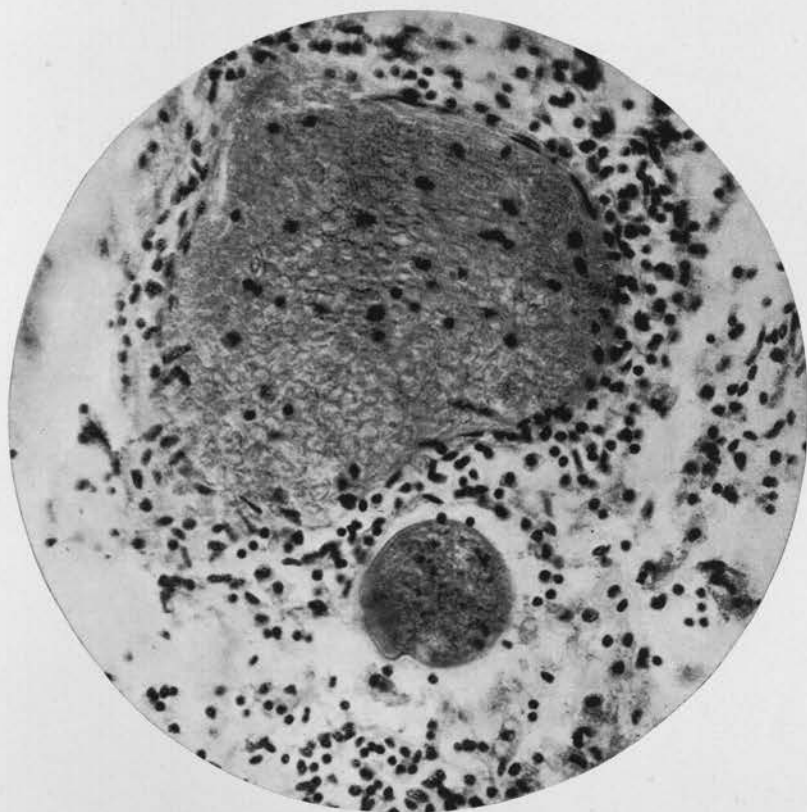


PLATE V. SECTION OF MESENTERIC LYMPH GLAND OF MONKEY 31, SHOWING A SINGLE BALANTIDIUM COLI HOMINIS IN THE CENTER OF THE GLAND.



PLATE VI. SECTION OF THE LARGE INTESTINE OF A MAN DEAD FROM BALANTIDIAL DYSENTERY.

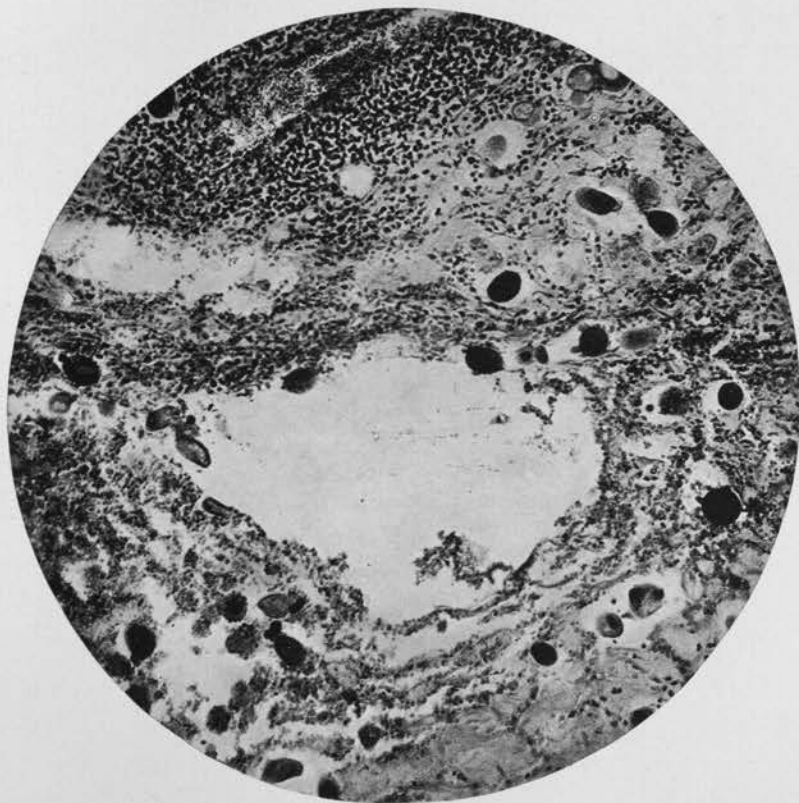


PLATE VII. SECTION OF THE LARGE INTESTINE OF A MAN DEAD FROM BALANTIDIAL
DYSENTERY.

THE INFLUENCE OF COMPENSATED SALT MIXTURES ON THE DEVELOPMENT OF POLYNEURITIS GALLINARUM AND BERIBERI

By R. B. GIBSON

(From the Laboratory of Physiology, College of Medicine and Surgery,
University of the Philippines)

Four plates

Recent work on the etiology of beriberi has shown that this disease develops because of the absence from the diet of some substance or substances necessary for the normal nutritive process of the body. Thus Strong and Crowell¹ have shown that the disease may occur in man under the most favorable hygienic conditions with exception in regard to diet. That beriberi in man may be caused by limited diets which do not include polished rice is evident from the observations of Axel Holst² on the occurrence of the disease on Norwegian ships; of Little³ on the existence of beriberi on the coasts of Labrador and Newfoundland, where white wheat flour is the chief article of diet in certain seasons; and finally of Lovelace⁴ that cases have occurred in Brazil. Beriberi, then, is a subject of world-wide interest. Furthermore, the study of this disease promises to do much toward clearing up some of the important problems in the physiology of nutrition.

The observations of Fletcher⁵ and of Fraser and Stanton⁶ have shown that diets consisting chiefly of polished rice are the common cause of beriberi in the Orient. If the white rice, however, was replaced by the rough rice, the disease did not develop. Substitution of rough rice for the white article and additions to the dietaries of the native military forces and public institutions in the Philippine Islands have eliminated beriberi

¹ *This Journal*, Sec. B (1912), 7, 271.

² *Journ. Hyg.* (1907), 7, 619; *Trans. Soc. Trop. Med. & Hyg.* (1911), 5, 71.

³ *Journ. Am. Med. Assoc.* (1912), 58, 2029.

⁴ *Ibid.* (1912), 59, 2134.

⁵ *Lancet* (1907), 1, 1776.

⁶ *Ibid.* (1909), 1, 451; *Studies from Institute for Medical Research. Federated Malay States* (1909), No. 10.

from these organizations.⁷ Shibayama,⁸ however, states that Japanese laborers, eating fresh unpolished rice, have developed the disease; and Strong and Crowell⁹ have observed mild symptoms of beriberi in men fed chiefly on Philippine red rice and also on white rice with an added alcohol extract of the polishings. Schaumann,¹⁰ in the course of an extensive study, found that the addition of dried egg white to maize fed to 3 rabbits did not prevent the development of polyneuritis. Eight doves receiving rice, desiccated egg white, and sodium chloride died even before the controls. Five doves were fed on boiled rice to which had been added 1 per cent of a mixture made up of potassium carbonate, 30 grams; sodium chloride, 3 grams; anhydrous sodium sulphate, 2 grams; calcium carbonate, 3 grams; magnesium carbonate, 4 grams; and iron oxide, 3 grams; no protective action was obtained. Grijns¹¹ had previously found that sodium or iron carbonate and that Röhmann's¹² salt mixture were without effect in beriberi. Schaumann also tried the effect of various compounds containing phosphoric acid. Calcium phosphate, glycerophosphate, phytin from rice polishings and yeast nucleic acid, and phosphatids were without, or had but slight, protective properties. However, yeast, testicle, rice polishings, mongo beans, peas, and bran prevent the development of polyneuritis. Schaumann concluded that in as much as these substances are rich in phosphoric acid the absence of some phosphorus-containing substance in the diet is responsible for beriberi. Similar conclusions are reached from the consideration of the composition of the diets of sailors who were suffering from ship beriberi and from the analyses for phosphates, sulphates, and urea in the twenty-four-hour urines of these patients in the course of therapeutic feeding with the above substances. In the experiments, Schaumann employed doves, rabbits, guinea pigs, rats, dogs, monkeys, and goats.

Fraser and Stanton¹³ studied the relation of the phosphoric acid content of rice to beriberi production for different rice samples. Using fowls¹⁴ which they found do not develop polyneuritis when fed on rough rice or on white rice to which the

⁷ Chamberlain, *This Journal*, Sec. B (1911), 6, 133; Heiser, *ibid.* (1911), 229.

⁸ *Ibid.* (1910), 5, 122.

⁹ *Loc. cit.*

¹⁰ *Beih. z. Arch. f. Schiffs- u. Trop.-Hyg.* (1910), 14, 325.

¹¹ Cited from Schaumann, *loc. cit.*

¹² *Allg. med. Zentr.-Zeitg.* (1903), No. 1; (1908), No. 9.

¹³ Studies from Institute for Medical Research. Federated Malay States (1911), No. 12.

¹⁴ Eijkmann, *Virchow's Arch.* (1897), 148, 523.

polishings had been added, they showed that a definite relationship actually existed. The total phosphoric acid content may be used as an indicator of the extent to which rice has been milled or polished, and therefore of its beriberi-producing qualities. These investigators further showed that the protective principle in the rice polishings was soluble in alcohol or in 0.3 per cent hydrochloric acid. Phytin, which comprises 32.5 per cent of the substances soluble in these reagents, was without protective properties.

Aron and Aron and Hocson¹⁵ found that nitrogen equilibrium could be maintained on a diet consisting chiefly of white rice in metabolism experiments lasting over a few days. The phosphorus balance became positive in beriberi cases or in the normal metabolism experiments when rough rice, rice polishings, or phytin were added to the dietary. They also examined the phosphorus content of 28 samples of rice, for the most part native, and found that the phosphorus content within narrow limits is determined by the grade of milling. Teruuchi,¹⁶ however, has recently concluded that the phosphoric acid metabolism is not altered in beriberi.

From a consideration of the diet on which beriberi developed among the Philippine Scouts, Kilbourne¹⁷ suggests that the disease may be due either to deficient phosphates or to a disproportion of calcium and magnesium. Chamberlain, Bloombergh, and Kilbourne¹⁸ concluded that the food supplied was deficient in potassium and phosphorus. Furthermore, analyses of white and unpolished rice showed that the latter contained about twice as much phosphoric acid and two and one-half times the potassium as did the former. In experiments with fowls, the addition of potassium chloride, of phosphoric acid, or of both was without effect. Starvation experiments induced neuritis as evidenced chiefly by histological examination.

Chamberlain and Vedder¹⁹ fed each of 4 groups of fowls on white rice, plus 0.07 gram of potassium diphosphate, potassium citrate, potassium carbonate, and magnesium phosphate, respectively. This amount was used because it is slightly in excess of the amount of each of these salts present in 5 grams of rice polishings, which are protective. The fowls developed neuritis. These authors also found that the residue of the alcoholic extract

¹⁵ *This Journal*, Sec. B (1910), 5, 81; *ibid.* (1910), 98; *ibid.* (1911), 6, 361.

¹⁶ *Verhandlungen der Japanischen Pathologischen Gesellschaft* (1912), 32.

¹⁷ *This Journal*, Sec. B (1910), 5, 127.

¹⁸ *Ibid.* (1911), 6, 177.

¹⁹ *Ibid.* (1911), 251.

of rice polishings, when taken up in water, contains practically no phosphoric acid compounds, but still prevents polyneuritis. The protective substance was capable of dialysis through a semi-permeable membrane. The same authors²⁰ confirm the earlier observations of Pol²¹ that extract of the mongo bean is protective.

Wieland²² reports analyses of mice which had been fed on rice. He found that such animals were no poorer in phosphorus than the controls. It would seem from these experiments that the etiology of the disease was not associated with deficiency in the phosphorus supplied to the organism.

Andrews²³ has made some very interesting observations on infantile beriberi. Analyses were made of the milk obtained from 11 women whose infants had died of beriberi, as confirmed by necropsy. The figures show the milk in these cases to be scant, but some of them seem normal so far as protein, fat, and carbohydrate are concerned. Both the calcium and phosphoric acid content of the samples analyzed were above the normal. Puppies, allowed to suckle these women, died. Symptoms and necropsy agreed entirely with those of the infants dying of infantile beriberi.

Chamberlain, Vedder, and Williams²⁴ found that arginine, histidine, asparagine, and other amino-acids, lipoids of the lecithin group, choline, and extract of onions were without protective action against polyneuritis in fowls. The neuritis-preventing principle was shown to be insoluble in ether. In a subsequent paper, Vedder and Clark²⁵ state that fowls receiving 10 grams of meat or potatoes or 5 cubic centimeters of cow's milk per day, with polished rice, receive partial protection only. Peas or peanuts given with the rice prevent the development of polyneuritis.

Funk²⁶ has reported the apparent isolation by phosphotungstic acid precipitation of the alcohol-soluble protective substance from yeast, rice polishings, and other foodstuffs. He considers this substance a pyrimidine derivation of the composition $C_{17}H_{40}N_2O_7$ and melting at 233° . Suzuki, Shimanura, and

²⁰ *Ibid.* (1911), 395.

²¹ Cited from Schaumann, *loc. cit.*; cf. also Pol, *Arch. f. Schiffs- u. Trop.-Hyg.* (1910), 14, 63.

²² *Arch. f. exper. Pathol.* (1912), 69, 293.

²³ *This Journal, Sec. B* (1912), 7, 67.

²⁴ *Ibid.* (1912), 39.

²⁵ *Ibid.* (1912), 423.

²⁶ *Journ. Physiol.* (1912), 45, 75.

Odaki²⁷ also obtained a phosphotungstic acid precipitable protective substance from the alcoholic extract of rice polishings. Vedder²⁸ has not succeeded in isolating this substance in experiments as yet reported. In the same paper, Vedder reports incidentally experiments on fowls with rice and Röhmman's salt mixture which are negative. The paper appeared while the present work was under way. Vedder and Clark²⁹ suggested the occurrence of at least two "vitamines" in the alcoholic extracts of the rice polishings to account for the types of polyneuritis gallinarum which they distinguished.

Osborne and Mendel³⁰ have recently published a comprehensive study of the rôle of the individual proteins in nutrition. They have followed the growth changes in young rats when fed on a single highly purified protein, along with fat, carbohydrate, and a salt mixture having the same composition as the salts in milk. The experiments show that normal growth can occur only when the protein component of the diet is complete as regards the amino-acids obtained on cleavage. Mere maintenance, or even nitrogen starvation, results when an incomplete individual protein is fed. The food in Osborne and Mendel's experiments contains no purines or nucleic acid. In the case of the vegetable proteins, such as edestin, there is no organic phosphorus, yet normal growth was obtained even with a diet consisting of edestin, starch, sugar, and salts. There is apparently no physiological necessity for organic phosphorus, lipid, or organic iron (in more stable form than the citrate).

According to Osborne and Mendel, then, provided sufficient calories are fed as fat or carbohydrate, but two dietetic factors are necessary—a chemically complete protein and a physiologically balanced salt mixture. It would seem that the development of polyneuritis in fowls ought to be prevented when white rice is fed with properly balanced mineral ingredients, provided the rice protein is nutritively adequate.

Deficiency in the protein may be excluded. The chief protein of the rice³¹ is oryzenin, a glutelin. Suzuki, Yoshimura, and Fujii³² have determined some of the amino-acid cleavage products of this protein. Their analyses indicate that the protein is

²⁷ *Biochem. Zeitschr.* (1912), 43, 89.

²⁸ *This Journal, Sec. B* (1912), 7, 412.

²⁹ *Loc. cit.*

³⁰ *Zeitschr. f. physiol. Chem.* (1912), 80, 307; *Journ. Biol. Chem.* (1912), 12, 81; *ibid.* (1912), 473; *ibid.* (1912), 13, 233.

³¹ Kajiura, *Biochem. Journ.* (1912), 6, 171.

³² *Journ. College Agr., Imp. Univ. Tokyo* (1909), 1, 77.

to be regarded as "complete," although the presence of tryptophane was not determined. However, I have prepared some oryzenin, and found that it gives the Hopkins-Cole reaction. Karl Thomas³³ claims to have shown, too, that the rice proteins have over four-fifths the nutritive efficiency of casein.

Analyses of polished rice show a varying ash content of from about 0.45 to 1 per cent, although proportionally the mineral ingredients are fairly constant.³⁴ For comparison with Osborne and Mendel's experiments, I have calculated the inorganic constituents per kilogram of rice as follows:

TABLE I.—Salts per kilogram of food.

Constituent.	Osborne and Mendel's experiments.	Rice I.	Rice II.
	Grams.	Grams.	Grams.
Ash		*0.45	*1.03
Ca	5.9	0.12	0.20
Mg	0.7	0.30	0.74
Na	6.1	0.18	0.23
K	8.0	0.80	1.82
PO ₄	10.0	3.20	6.68
Cl	12.4	0.004	0.09
SO ₄	0.9	0.03	0.066
Fe	0.13	0.04	0.11
Citric acid	10.0		
SiO ₂	0.0	0.12	0.64

* Per cent.

Most striking differences are observed. The rice is deficient in calcium, and there is, relative to the element, a much greater proportion of magnesium. Sodium and potassium chloride are low. The total amount of the mineral constituents is low. The salts certainly seem far from being properly balanced to maintain normal physiological activity over relatively long periods of time. The presumable absence of mineral salts of the organic acids, in the white rice, might be considered as a contributing factor to the development of beriberi; particularly is this point significant for polyneuritis gallinarum, since the end product of nitrogenous katabolism, uric acid, is eliminated as the urate. In as much as certain salts of the organic acids are soluble in alcohol, part of the protective effects of the alcoholic extracts of

³³ Arch. f. Anat. u. Physiol. (1909), 219.

³⁴ Kellner, Uchiyama, and Yamada, *Die landw. Versuchsstationen* (1892), 41, 295.

the rice polishings may be due to this factor. In fact, qualitative tests have shown that calcium, potassium, and some organic acid, other than lactic acid, are present in such extracts of the rice polishings.

It is probable that the balanced inorganic constituents of Osborne and Mendel's ration are not as well adapted for fowls as for mammals. Still, in the experiments reported in this paper, the attempt has been made to supply the several salts to the rice fed to fowls with particular reference to calcium and the salts of some organic acid. While the experiments have not been successful in this respect, the results are of sufficient interest to warrant publication. Some experiments with monkeys, one of which developed an almost typical case of beriberi, are included.

PREPARATION OF THE SALT MIXTURES

The rice employed (Philippine No. 1) had an ash content of 0.47 per cent. A stock salt mixture was made with rice flour, but otherwise prepared in essentially the same manner as described by Osborne and Mendel. When the stock salt mixture was added to about two and one-half times the weight of cracked rice, 1 kilogram of the food would have approximately the composition for the mineral ingredients recommended by Osborne and Mendel if the analyses for "Rice I" can be taken as representative. In the calcium experiments, the carbonate was neutralized with hydrochloric acid or with both hydrochloric and lactic acids (the latter in amount equivalent to the citric acid in Osborne and Mendel's experiments). In two cases, half the above amount of lactic acid, as sodium lactate, was fed with the polished rice.

For 980 grams of rice flour there were used CaCO_3 , 53 grams; MgCO_3 , 5.5 grams; K_2CO_3 , 50.8 grams; Na_2CO_3 , 54.3 grams; Fe-citrate, 1.7 grams; HCl (sp. gr. 1.20), 137.7 cubic centimeters; H_2SO_4 (sp. gr. 1.84), 2 cubic centimeters; H_3PO_4 (85 per cent), 19.3 cubic centimeters; and citric acid, 40 grams. In the calcium chloride experiments 54 grams of CaCO_3 were neutralized with HCl and added to 1 kilogram of rice flour; and for the lactate experiments a mixture of 30 grams of CaCO_3 neutralized with lactic acid and of 24 grams of CaCO_3 neutralized with HCl , per kilogram of rice flour, served as the stock salt mixture.

EXPERIMENTAL METHODS

Well-grown young male fowls were used in the experiments. These were kept in individual cages with alberine stone floors and provided with a perch. The cages were cleaned daily. In the earlier experiments, the stock rice-salt mixture was made

into a granulated paste with cracked white rice and water, and the amounts eaten by each fowl recorded. In subsequent experiments, the fowls were fed when they refused to eat the rice provided. It was found that they would eat voluntarily sometimes as much as 85 grams of rice per day, although many fowls may be maintained upon an average of 35 grams of rice. The chickens were weighed every morning before feeding, and the condition of each noted. Fowls dying were necropsied, and the sciatic nerve removed to study the degenerative changes, in as much as Clark has stated that microscopic evidence of polyneuritis may be observed even a week after the rice feeding has been started.

THE EFFECTS OF FEEDING WHITE RICE WITH THE COMPENSATED SALT MIXTURE

Three fowls were fed on the white rice used in the subsequent experiments for controls. These developed polyneuritis on the nineteenth, twenty-second, and twenty-ninth days, respectively. Examination of the sciatic nerves by the Marchi method showed typical and pronounced Wallerian degeneration in each case.

Three more fowls were fed on white rice plus the stock salt preparation. These developed neuritis on the thirty-seventh, thirty-seventh, and thirtieth days, respectively. Degeneration was typical in certain fibers in one case, typical but not pronounced in the second, and very pronounced in the third instance.

The experiment is summarized in Table II.

TABLE II.—*Influence of feeding rice and mixed salts.*

No.	Nature of experiment.	Weight of fowl on the—					Result.
		First day.	Seventh day.	Twenty-first day.	Twenty-eighth day.	Thirty-fifth day.	
1	Control	960	993	943			Neuritis, nineteenth day. Degeneration + + +.
2	do	1,174	1,210	1,125	1,022		Neuritis, twenty-second day. Degeneration + + +.
3	do	985	1,081	1,005	1,060		Neuritis, twenty-ninth day. Degeneration + + +.
4	Salt mixture	1,084	1,052	942	940	980	Neuritis, thirty-seventh day. Degeneration + +.
5	do	1,155	1,000	1,065	1,090	1,090	Neuritis, thirty-seventh day. Degeneration +.
6	do	1,130	1,176	1,099	1,040		Neuritis, thirtieth day. Degeneration + + +.

The addition of the compensated salt mixture, then, has not prevented the development of polyneuritis in fowls. In the experiments reported above, however, the onset of the disease seems to have been slightly protracted, and the degenerative changes in the nerves were less pronounced than in the controls.

EXPERIMENTS WITH CALCIUM CHLORIDE AND WITH LACTATES

As has been stated, as compared with Osborne and Mendel's salt mixture, the rice is notably deficient in calcium. Accordingly, 3 pairs of fowls were fed on rice with the addition of the calcium lactate and calcium chloride, and with the calcium chloride alone.

One fowl, allowed to eat voluntarily, progressively increased in weight from 1,035 to 1,212 grams on the calcium lactate and calcium chloride mixture, but on the fiftieth day developed neuritis. Histological examination of the sciatic nerve showed typical degeneration in a few nerve fibers, along with *many nuclei of the embryonic or regenerating type in the fiber sheath*. (These histological findings and their significance for the question of regenerative changes in nerve fibers are to be discussed by Clark in a paper shortly to be published.) The other fowl of this pair, which received the calcium chloride rice only, gradually dropped in weight from 1,165 to 744 grams, and died of general physical weakness, there being *no symptoms of neuritis*. Histological examination of the sciatic nerve gave only one or two fibers showing the typical degeneration. These and the other experiments are given in Table III.

The calcium lactate-chloride fowl of the second pair lost weight after the second week, developed chicken pox on the thirty-second day of the experiment, and practically recovered from this but was found dead on the forty-seventh day. There were no symptoms of neuritis. At necropsy, numerous nodules were found in the wall of the intestine, which on section seemed to inclose some animal parasite. Histological examination of the right sciatic nerve showed fibers in the preparation typically degenerated. The calcium chloride fowl gradually fell in weight from 1,076 to 940 grams developing chicken pox on the twenty-seventh day, when the daily weight fell rapidly. This fowl practically recovered from the pox, but gradually grew weaker, and was killed on the thirty-sixth day. Neuritis was questionable, and probably had not developed. Only a very few fibers of the sciatic showed the Wallerian degeneration. Both the above chickens were allowed to eat voluntarily.

The third pair of fowls was given 60 grams of the calcium rice mixture per day, and, when the food was unconsumed, was fed the balance. The calcium lactate-chloride fowl gained rapidly in weight from 1,170 to 1,295 grams on the twenty-first day of the experiments; then the weight dropped slightly, and again rose to 1,260 grams on the thirty-second day of the experiment, when neuritis developed. Examination of the sciatic nerve showed moderate although typical degeneration. The calcium chloride fowl of this pair gained in weight from 1,167 to 1,300 grams for the first two weeks, and then gradually lost weight, seemed sick on the twenty-first day of the experiment, and died on the twenty-fifth day. Neuritis symptoms were questionable, and the sciatic fibers showed but little degeneration. It is probable that these two fowls received too much calcium, in as much as they were forced to consume almost twice the food per diem as was eaten by the other two pairs after the first few days of the experiment; there was, however, no evidence of hæmorrhages into the tissues of the œsophagus between the crop and the muscular stomach or of ulceration of the mucosa of the latter, which I have observed in chronic calcium poisoning in some feeding experiments not reported in this paper.

Two fowls were given one-half the amount of lactic acid fed in the above experiments, but in the form of sodium lactate. One fowl gained rapidly in weight from 1,055 to 1,200 grams on the twentieth day of the experiment, often voluntarily eating 75 grams of rice per day. The body weight did not fall below 1,150 grams until the thirty-seventh day of the experiment, when he ate little and seemed sick. The salt mixture was changed so that the fowl received twice the former amount of lactic acid, half as sodium and half as the calcium lactate, and half the amount of calcium chloride as was received by the calcium lactate-chloride chickens in the above experiments. He was given, or fed in part, 60 grams of this rice and salt mixture per day, and rapidly improved in weight and condition. On the sixtieth day, when the fowl weighed 1,187 grams, incipient neuritis was evident. The fowl was killed on the sixty-second day. Examination of the sciatic nerve showed most profound degeneration, nearly every fiber being involved. Numerous nuclei of the embryonic nerve fiber type were found in histological preparations of the sciatic. The second sodium lactate fowl was started simultaneously with the other, and rapidly gained from 1,040 to 1,160 grams the first week. Subsequently, he ate much less, and the body weight dropped to 1,047 grams on the twenty-first day.

When fed by hand, the weight was increased to 1,092 grams on the thirtieth day; the fowl, however, seemed slightly sick, and died suddenly on the thirty-third day from some cause not revealed at necropsy. There was no evidence of degeneration on histological examination of the sciatic nerve.

A striking incidental observation is found in that the fowls, which received the lactate, developed brilliant red erect combs and wattles and a fine plumage. This is in marked contrast with the results obtained in the other rice-fed fowls. These differences are shown in the accompanying reproductions of photographs of the chickens.

The fact that the lactate chickens have not lost weight, and in particular have put on weight even when the experiments have extended over relatively long periods, is important. This finding suggests that a closer symptomatic relationship exists between polyneuritis gallinarum and beriberi in man than the evidence heretofore available has permitted to be accepted.³⁵ From these experiments, it would seem that the administration of calcium salts or of lactates has prolonged the period required for the development of the polyneuritis. Furthermore, it seems that the fowls will not survive for long the administration with the rice of the calcium as the chloride only. Regeneration processes, as evidenced by the presence of embryonic nuclei in the nerve fibers of one fowl receiving calcium lactate and of a second on sodium lactate and then calcium lactate, are most suggestive.

EXPERIMENTS WITH MONKEYS FED ON WHITE RICE AND SALTS

Schaumann³⁶ found that a monkey, fed on rice, lost appetite, and developed a paralysis of the lower extremities and progressive marasmus. Degeneration of many nerve fibers was evident. Aron³⁷ obtained a somewhat similar result with 3 monkeys fed on white bread. Shiga and Kusama³⁸ observed in a monkey at first an increase of appetite followed after thirty-seven days by a loss of appetite, and subsequently by a loss of the patellar reflex and paralysis of the lower extremities. The animal died ten days later. There was degeneration of the peripheral nerves and the cells of the anterior horn, atrophy of the musculature, etc.; the heart showed a dilatation hypertrophy. Nagayo and

³⁵ Cf. Shibayama, *loc. cit.* Eijkman, *Arch. f. Schiffs- u. Trop.-Hyg.* (1911), 15, 65; Clark and Vedder, *loc. cit.*

³⁶ *Loc. cit.*

³⁷ *Loc. cit.*

³⁸ *Loc. cit.*

TABLE III.—*Rice fed with CaCl₂, Ca-lactate, and Na-lactate.*

No.	Material fed.	Weight of fowl on the—									Result.
		1st day.	7th day.	14th day.	21st day.	28th day.	35th day.	42d day.	49th day.	56th day.	
		<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	<i>Grms.</i>	
1	Ca-lactate.....	1,035	1,058	977	1,080	1,079	1,105	1,200	1,212	-----	Neuritis fiftieth day. Degeneration ++, but showing embryonic nuclei.
2	Ca-chloride.....	1,165	1,025	900	810	744	-----	-----	-----	-----	Died, thirtieth day. Degeneration +.
3	Ca-lactate.....	1,088	1,075	974	1,010	1,013	^a 942	932	-----	-----	Died, forty-seventh day. Degeneration ++.
4	Ca-chloride.....	1,076	1,014	989	940	^a 789	709	-----	-----	-----	Killed, thirty-sixth day. Neuritis? Degeneration +.
5	Ca-lactate.....	1,170	1,225	1,267	1,295	1,242	-----	-----	-----	-----	Neuritis, thirty-second day. Degeneration ++.
6	Ca-chloride.....	1,167	1,195	1,267	1,180	-----	-----	-----	-----	-----	Died, twenty-fifth day. Neuritis? Degeneration +.
7	Na-lactate.....	1,055	1,150	1,181	1,177	1,170	^b 1,165	1,155	1,183	1,168	Neuritis, sixtieth day. Degeneration +++ +, but showing embryonic nuclei.
8	do.....	1,040	1,152	1,094	1,047	1,067	-----	-----	-----	-----	Died, suddenly, thirty-third day. Neuritis? Degeneration not evident.

^a Mild attack of chicken pox.^b Food changed from Na-lactate to half Na-lactate and half Ca-chloride and lactate rice.

Fujii³⁹ fed 6 monkeys on cooked white rice. Of these, 2 died of simple inanition, 3 of inanition with scorbutic changes, and the sixth in thirty-four days of inanition with symptoms of beriberi. In the sixth monkey the pain sense and patellar reflex were depressed but present during the last days; the heart was somewhat dilated, but not hypertrophied to any great extent; the lungs were congested and œdematous, and there was degeneration of the cells of the anterior horn of the cord and of the peripheral nerves.

Six recently trapped monkeys of the common Philippine species, *Pithecus syrichta* (Linnæus), were obtained and placed in separate cages. These were fed on rice which had been boiled until soft in a relatively large amount of distilled water; the rice was washed two or three times, and the water strained off through gauze. To the rice fed to three of the monkeys there was added a salt mixture made up according to the method Osborne and Mendel, except that rice flour was used instead of the lactose. A little banana was added to the rice when cooked to flavor it, as it was found that the monkeys were refusing to eat after the first few days of the experiment.

On the forty-second day of the experiment, 1 of the salt-fed monkeys, whose weight had fallen from 1,341 to 1,289 grams, became œdematous. The œdema was especially noticeable in the face. On the following day the œdema was more striking. The third day the œdema had largely disappeared, but the monkey was evidently sick, and was irritable when touched. On the forty-sixth day of the experiment, the animal was lying on its side and evidently dying. At necropsy, the body seemed poorly nourished, with the viscera normal except for a slight gastritis. The right heart was greatly dilated, the ventricle wall being very thin. The heart appeared as if double apexed. The lungs seemed normal. There was no excess of fluid in the body cavities, but the tissues seemed wet when cut. Histological examination of the sciatic nerve, as may be seen in the accompanying photomicrograph, showed typical Wallerian degeneration (Plate IV).

The other 5 monkeys became progressively marasmic. There was no marked difference in the development of the conditions in the salt-rice and the rice groups. One of the monkeys fed on rice alone died on the eighty-sixth day of the experiment. At necropsy the monkey was found to be rough-haired, very poorly nourished and apparently starved, somewhat jaundiced,

³⁹ Verhandlungen der Japanischen Pathologischen Gesellschaft (1912), 39.

but with no obstruction in the common or cystic duct, and without any special evidence of beriberi. The sciatic nerve, however, was found on histological examination to show typical degeneration.

The experiment was discontinued after one hundred twenty days, when all the remaining animals, then in very bad condition, were returned to a mixed diet.

It has been shown that the attempt to keep monkeys on the salt mixture added to water-extracted rice has not sufficed either to maintain the weight of the animals or to prevent the development of an almost typical case of beriberi in one instance. The experiments again serve to show the resistance of monkeys to white rice as compared with man.

SUMMARY AND CONCLUSIONS

The addition of a compensated salt mixture to white rice fed to fowls has not prevented the development of polyneuritis gallinarum. However, the onset of the disease seems to have been slightly protracted, and the degenerative changes in the nerves were less pronounced.

Fowls fed on white rice and lactates, and in particular calcium lactate, have maintained body weight, even when the experiments have extended over relatively long periods. This fact suggests that a closer symptomatic analogy may exist between polyneuritis gallinarum and beriberi in man than the evidence available has hitherto permitted to be drawn. The administration of calcium salts or lactates prolonged the period required for the development of neuritis, though the fowls did not long survive the diet of rice and calcium chloride. The lactate-fed fowls developed brilliant combs and a fine quality of plumage in contrast with all other chickens employed in these experiments. Regenerative processes, as evidenced by the discovery of embryonic nuclei, were suggested on examination of the nerve fibers of 2 fowls which had received calcium lactate.

The attempt to keep 3 monkeys on an approximately compensated salt mixture and white rice failed either to maintain the body weight of the animals or to prevent the development of an almost typical case of beriberi in 1 case. These experiments, with the 3 controls, demonstrate again the resistance of monkeys to white rice as compared with man.

Osborne and Mendel have shown that, with an exactly physiologically balanced salt mixture, rats may maintain their weight and even grow to maturity when fed on pure protein with carbohydrate or carbohydrate and fat. However, it is evident that

under normal circumstances of life such conditions do not obtain for the individual except in the early stages of its development. Accessory factors must play a rôle of extreme importance in normal dietaries, as shown by Hopkins ⁴⁰ who found that a given increment of growth in young rats is attained with much greater economy when a ration of milk is added to the artificial mixture of casein, carbohydrate, lard, and salts. The feeding experiments which I have carried on again emphasize the existence of such accessory factors for normal nutrition.

Since this paper was written, Clark has made a study of the so-called "embryonic nerve fiber." The results are in part based on the examination of the nerves of the "calcium lactate" fowls and a full description of these nerves will be given in Clark's paper. He concludes that the "embryonic nerve fiber" is a stage of advanced degeneration rather than a regenerative phenomenon.

I have shown that the calcium lactate has prolonged the onset of symptoms of the polyneuritis. Clark believes that the longer course of the disease has permitted degeneration to occur in certain fibers of the sciatic to a degree much more intense than has hitherto been observed for fowls fed on rice alone; for instance, a degeneration of the type observed after a nerve is cut. The ordinary rice neuritis, then, must be of a relatively mild type, as is further evidenced by the rapid recovery, often in two or three hours (Funk), subsequent to the administration of the "vitamine" preparations.

⁴⁰ *Journ. Physiol.* (1912), 44, 425.

ILLUSTRATIONS

(Photographs by Cortes)

PLATE I

- FIG. 1. Fowl fed on the rice and calcium chloride, after thirty days.
2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE II

- FIG. 1. Fowl fed on the rice and calcium chloride, after thirty days.
2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE III

- FIG. 1. Fowl fed on 60 grams of the rice and calcium chloride per day, after thirty days.
2. Fowl fed on 60 grams of the rice and calcium lactate and chloride per day, after thirty days.

PLATE IV

Microphotograph of the sciatic nerve of the monkey which died with beriberi symptoms after forty-six days on a diet of boiled rice with the salt mixture (Marchi preparation).



Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.

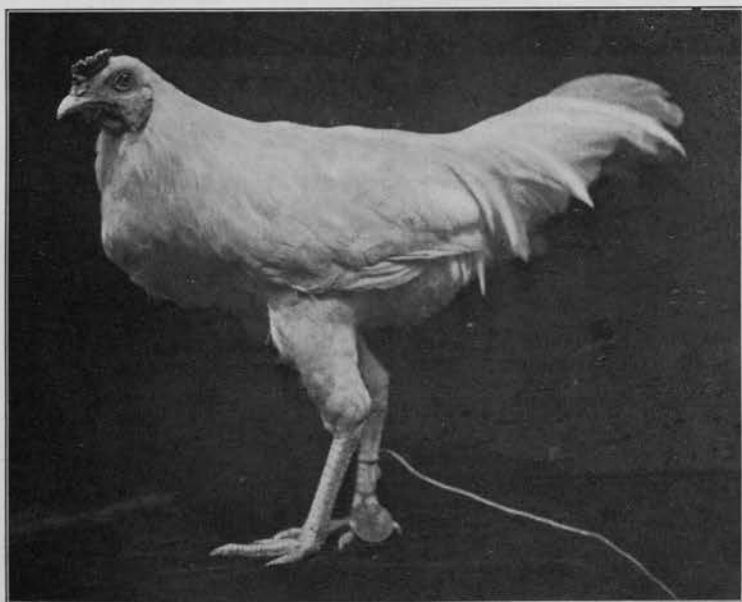


Fig. 2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE I.

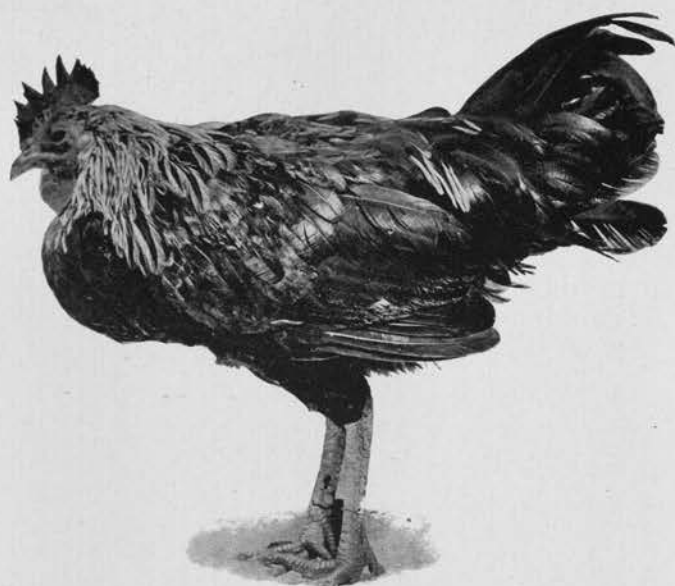


Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.

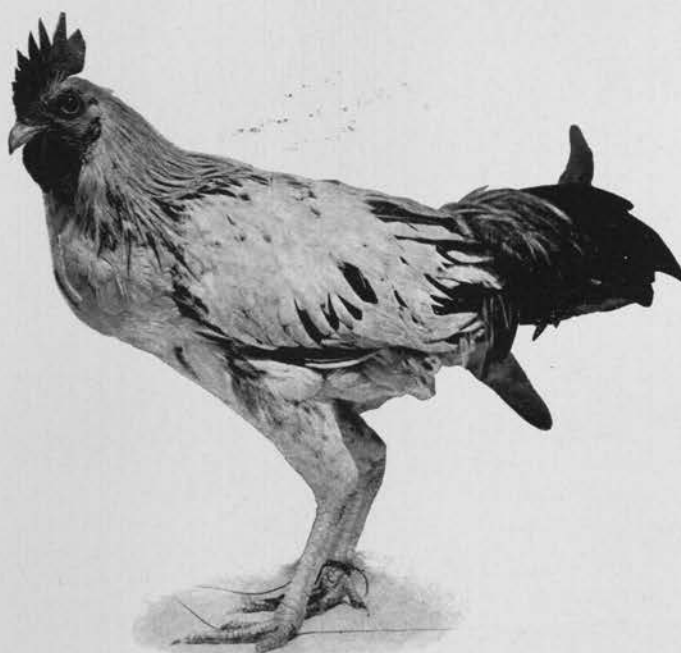


Fig. 2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE II.

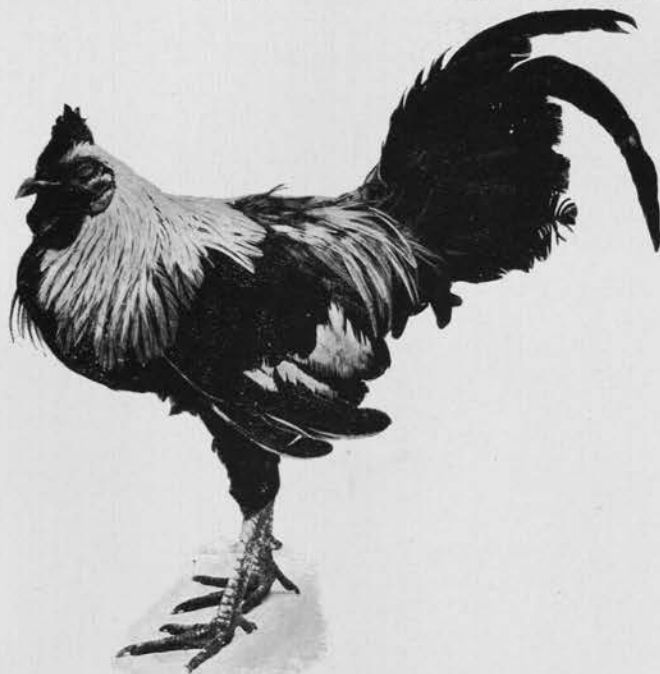


Fig. 1. Fowl fed on 60 grams of the rice and calcium chloride per day, after thirty days.



Fig. 2. Fowl fed on 60 grams of the rice and calcium lactate and chloride per day, after thirty days.

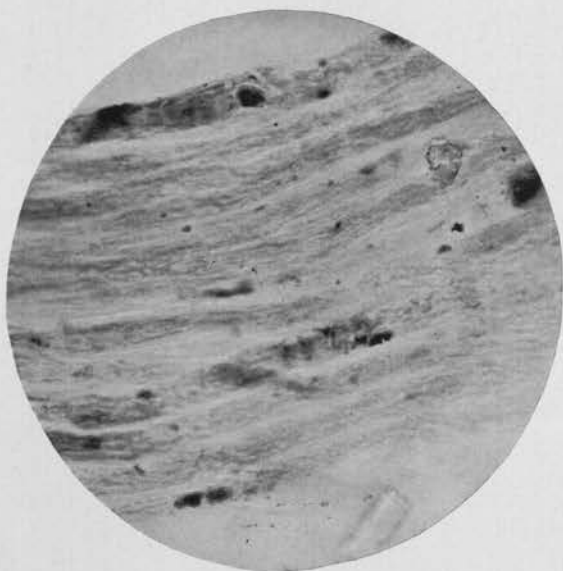


PLATE IV. MICROPHOTOGRAPH OF THE SCIATIC NERVE OF THE MONKEY WHICH DIED WITH BERIBERI SYMPTOMS AFTER FORTY-SIX DAYS ON A DIET OF BOILED RICE WITH THE SALT MIXTURE (MARCHI PREPARATION).

AN UNUSUAL DISEASE PREVAILING IN EPIDEMIC FORM AT BUHI, AMBOS CAMARINES, P. I.

By MARSHALL A. BARBER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

On October 17, 1912, Dr. Segundo Isaac, acting district health officer of Ambos Camarines, reported the presence of an unusual disease prevailing at Buhi, Ambos Camarines Province, Luzon. His report, in summary, is as follows:

Eight deaths were reported to me as caused by this disease from August 15 to date. It is suspected that some of the deaths were due to other diseases. Of these 8 fatal cases, 5 were thoroughly investigated:

Zeila Juarez: A girl of 13 years. The morning of October 3 she noticed a red tumor on her breast. This tumor was hot, somewhat hard, and gave very little pain. On the next day she felt slight pain in it. She died on October 5 with subnormal temperature.

Maria Sabinorio: A girl 14 years of age. She felt slight pain in the breast on the morning of October 6, the pain extending later in the day to the stomach. Little pain was felt until her death the next day at 2 o'clock in the afternoon.

Justa Balectora: On September 26 she was pricked by a small piece of bamboo. Inflammation of the part followed. The lesion was soft and white in the center with some infiltration and inflammation. On the 28th inflammation extended to the forearm and the elbow. At night she suffered headache and pain in the bowel. She died on the morning of the 30th, not suffering great pain.

Severino Olicerres: On October 4 he was pricked on the hand by a bamboo thorn. A slight inflammation followed which disappeared after a little treatment. The night of October 7 a swelling appeared on the breast, not painful but somewhat hard and red. The next day the patient felt a slight pain in the bowel, and died at 3 o'clock in the afternoon.

Esperanza Arcilla: A girl 2 years of age. She had a very small nodule in the leg. It was cauterized, and disappeared; but after a while another nodule appeared near it. This was treated the same way and also disappeared, but was followed by a third in the same region. After two days she died with the same symptoms as the above-mentioned cases.

Other patients suffering with this disease are likely to recover. In almost all of the fatal cases the patients died after a very short illness and with very little pain. The disease begins in the skin, extends to other parts of the body, and death follows with subnormal temperature.

Almost all the people of the town declare that this disease is, in all respects, similar to the disease that is causing great mortality among carabaos.

As a measure for its suppression, instructions were given to the president for the isolation of the infected persons and the disinfection of the premises.

It is recognized that the provisional explanation of the cause of the Buhi epidemic is unsatisfactory, and the pyogenic bacteria may be associated with some other agent which is the real cause of the disease. However, it seemed worth while to give a brief account of the observations made by Doctor Isaac and by me, in the hope that they may be of some use to those who have seen, or may encounter, a similar disease.'

'It may be of interest to note the nature and variety of remedies used for this disease by the people of the village. Application of carbolic acid, petroleum, silver nitrate, tincture of iodine, and charcoal of coconut shell; and poultices of garlic, mustard, and of the leaves of the following plants; *anonang*, betel-nut palm, *dao* tree, cotton, and *calumpinag*. Also cupping with a cow's horn was practiced, burning with a lighted cigarette, and binding with a string to arrest the progress of the inflammation.

THE INFECTION OF ACHLYA WITH VARIOUS MICROÖRGANISMS

By MARSHALL A. BARBER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Three plates

In a previous paper¹ I described a method of inoculating microörganisms and other substances into the vacuoles or protoplasm of living cells; and, in a later article,² gave some of the results obtained by such inoculations into fish molds, various algæ, and the larvæ of a gnat. In this paper essentially the same method of inoculation has been used; but the host has been grown in pure culture, thus making it possible to add various media or other substances to the culture and to do away with bacteria other than the ones inoculated.

Achlya, one of the fish molds, was chosen for these experiments because of its large filaments and ease of cultivation.

Pure cultures were easily obtained by the method of single-cell isolation described by me in various publications.³ Insects were placed in water containing algæ, in the usual manner of obtaining cultures of fish molds. After a crop of zoöspores had formed, a mass of them in the resting condition was transferred to a hanging drop. With a fine capillary pipette, single zoöspores were separated from the mass, freed from bacteria by successive washings in droplets of sterile water, picked up with a fresh pipette, and each one transferred to a test tube containing distilled water plus a small quantity of glucose broth. The whole process of isolation of a series by this method requires only a few minutes. Or, a mass of zoöspores, partially freed from bacteria, may be deposited in one end of a long hanging drop of sterile water. These spores, at first invested with a membrane, burst the membrane within two or three hours and swarm to the other end of the drop. They may be picked

¹ *Journ. Infect. Dis.* (1911), 8, 348.

² *Ibid.* (1911), 9, 117.

³ *Sci. Bull., Kansas Univ.* (1907), 4, 3. *Journ. Infect. Dis.* (1908), 5, 380; (1909), 6, 634. An article describing the method in detail is now in preparation, and will be published in an early number of this Journal.

with the capillary pipette while swarming in the nearly or quite bacteria-free end of the drop, or after they have come to rest and germinated. Either method enables one to obtain a culture from a single zoöspore, but the latter sometimes facilitates freeing them from bacteria. Before finally transferring to the test tube, each isolated zoöspore may be examined in a small droplet of water with a higher power in order to make sure of the absence of bacteria.

In the absence of zoöspores or resting spores in the pure culture, one may make subcultures by transferring the mycelium grown in the liquid medium to sloped agar tubes. When growth is established there, new cultures may be made from detached pieces of agar containing living hyphæ.

For purposes of inoculation it is necessary to have a thrifty mycelium in a hanging drop of nutrient medium. For obtaining this there are several convenient methods.

From a young subculture growing in a thin layer of agar in a Petri dish one may cut with sterile knife or platinum spatula small blocks of agar from the margin where growth is most vigorous. These are transferred to the sterile cover glass, and, if desired, fresh nutrient medium is added to the margin of the block.

Another method would be by transferring bodily a small mycelium to a liquid medium in a hanging drop.

Zoöspores in this species were formed much less freely in pure culture than in ordinary water cultures with bacteria. But sufficient zoöspores for hanging drops or for making subcultures were obtained from a three to ten days' growth in test tubes containing about 10 cubic centimeters of distilled water with 0.05 to 1 cubic centimeter of 1 per cent glucose broth added to each tube. In one culture, glucose broth, made 5 + acid and added in the proportion of 0.1 cubic centimeter to 5 cubic centimeters distilled water, gave an abundant crop in from two to three days. Apparently some degree of starvation facilitates the formation of zoöspores in this species, since a vigorous growth in undiluted glucose broth or on sterilized insects in water usually failed to produce them.

The best mycelia for the purposes of these experiments were obtained from the large resting spores formed vegetatively at the tips of hyphæ. These usually occurred abundantly in old cultures in distilled water plus glucose broth. These are separated from the mycelium with the platinum loop, and when settled to the bottom of the test tube may be transferred by a pipette to a hanging drop. From this drop one or more are

selected and transferred to a second cover for the permanent culture. These spores produce in a few hours a thrifty mycelium with hyphae larger than those from zoöspores and more suitable for inoculation. Abundant zoöspores were obtained within twenty hours by sowing one of these resting spores in a hanging drop, consisting of the original fluid in which the resting spores had formed. This fluid was somewhat enriched by nutriment from the mycelium crushed in the process of separating the resting spores. No extended experiments were made to determine the conditions which favor the formation of various types of reproduction, since the primary object was simply to get material suitable for inoculation.

The following is an example of one series of isolations:

May 21. Resting zoöspores from the tip of a zoösporangium of *Achlya* were separated, washed, and transferred to tubes of distilled water plus a few drops of glucose broth. Tube 1 received 16 spores; tube 2, 6 spores; tube 3, 3 spores; tube 4, 4 spores; tubes 5, 6, 7, 8, and 9, each 1 spore.

May 23, all showed growth of *Achlya* except tube 8, which remained sterile. All were free from bacteria except tubes 1 and 2 which had received the masses of 16 and 6 spores. Tube 7 developed zoöspores after four days' growth at room temperature; but zoöspores could not be detected in any other. Five of the pure cultures, including tube 7, developed oogonia and antheridia after six days' growth, and the 2 contaminated ones developed them some time later. The addition of undiluted 1 per cent glucose broth to 2 tubes, 1 containing zoöspores the other none, brought about a luxuriant vegetative growth with few or no zoöspores or oogonia.

Both liquid and agar media were used for hanging drop cultures. In making these a large cover, about 38 by 65 millimeters in size, was sterilized and placed on the glass box used in isolating microörganisms. A rectangular or ovoid barrier of sterile paraffin was usually made on the underside of this cover by melted paraffin blown from the tip of a bent pipette. Within this inclosure the medium was placed, and the spore or mycelium planted in it. Such cultures may be grown for days over a moist chamber. This chamber should be 0.5 centimeter or more deep, else branches of the mycelium may grow to the bottom of the cell and introduce bacteria into the culture.

Liquid culture media may be readily withdrawn from such cultures and a new medium or any other liquid substance added by means of a sterile capillary pipette bent at the tip.

It is possible to inoculate a hypha so that all growth will

take place inside of the infected mycelium and none in the medium around it; or, if desired, to have one kind of organism growing in the host and another kind outside. To avoid inoculating the medium outside of the plant some special precautions are necessary. A hanging drop of the bacterium is made outside of the paraffin barrier, and beside it is placed a drop of sterile water or agar. After supplying the pipette with the necessary dose it is withdrawn from the bacteria and passed through the water or agar drop to remove from its surface any adhering bacteria. After inoculating the fungus the tip must be withdrawn very slowly and cautiously in order to prevent the forcing out of any of the injected bacteria by the cell pressure. In case some bacteria are left outside in the medium, it is often possible to remove them with an ordinary capillary pipette. If too numerous or scattered to be removed in this way, their growth may be restricted or their effects diminished by frequently withdrawing the old medium and substituting new, or by adding some specific serum unfavorable to the development of the bacterium.

In the following experiments, bacteria pathogenic to animals were inoculated in order to test their effects on plant protoplasm. Of motile forms, *Bacillus pyocyaneus* and the vibrios of Asiatic cholera were tested; and of the nonmotile, the bacillus of dysentery, Shiga-Kruse type, and the bacillus of bubonic plague.

With *Bacillus pyocyaneus* some 14 successful inoculations were made, the majority in pure cultures of *Achlya* in hanging drop at a room temperature of from 27 to 31° C. Under these conditions infection usually followed the inoculating of even very small doses, and the death of the filament occurred within twenty-four hours. Not only the hypha at the point of inoculation become infected, but any branches not plugged off from the inoculated filament soon became swarming to their finest endings with actively motile bacilli. Noninfected filaments often remained in good condition in the same hanging drop. The cause of the death of the infected filaments was, apparently, primarily due to exhaustion of nutriment by the bacteria, since the protoplasm of the host often remained living and motile when the bacilli were so numerous as to form practically an emulsion in the vacuole. *Bacillus pyocyaneus* grown outside the mycelium in an agar hanging drop may be tolerated two or three days.

After the death of the filament the bacteria continued to grow, packing the filament with densely crowded masses, and often bursting through the wall and forming masses usually at the tips of branches. (Plate I.) The whole infected filament takes

on a yellowish brown color and contrasts sharply with the surrounding healthy portion. The bacteria which burst out are in part living and may contaminate the medium.

Bacillus pyocyaneus is more rapidly destructive of the fungus than any other organism tested. The death of the infected filament may be delayed some days by keeping at refrigerator temperature, but the bacteria remain living and multiply on removal to a higher temperature.

Specific rabbit serum, agglutinating *Bacillus pyocyaneus* in a dilution of at least 1 to 200, immediately clumped bacilli outside the filaments, but had no effect on those inside, even in dead filaments where the cellulose wall of the host alone intervened between the serum and bacilli. Undiluted serum was discharged from a pipette against such walls with no effect on the motility of bacteria inside. This serum had no immediately harmful effect on healthy *Achlya* filaments.

Inoculation with the vibrios of Asiatic cholera gave results similar to those with *Bacillus pyocyaneus*, except that infection was less sure and proceeded more slowly. The contents of *Achlya* cells are evidently a favorable medium for the growth of cholera. This is shown not only by successful infection, but by the fact that vibrios lying outside are immediately attracted to a spot where the pricking of a hypha has allowed cell contents to escape.

An infected hypha after death became packed with vibrios in much the same way as with *Bacillus pyocyaneus*, and similar extrusions from burst places occurred, but less strikingly than in the case of *Bacillus pyocyaneus*. (Plate II.) Some 10 successful inoculations were made with cholera, all in *Achlya* cultivated in artificial media.

The experiments with agglutinating serum were repeated with cholera. Infected hyphæ, both dead and living, were treated with a rabbit serum agglutinating in a dilution of at least 1:500. Any vibrios outside were immediately clumped; but, as in the case of *Bacillus pyocyaneus*, the cellulose wall appeared to be an effective barrier against the passage of the agglutinins. In one experiment, a one-half dilution of the strongly agglutinating serum was injected directly into an infected hypha by means of a fine capillary pipette. The vibrios inside were immediately agglutinated, although they had been unaffected by the same dilution applied outside. The serum-injected hypha lived for at least three-fourths of an hour after the inoculation, as shown by the movement of its protoplasm. Infected cells, even though living and with actively motile protoplasm, had a diminished turgidity;

and it was much more difficult to inject a substance into them than into healthy cells.

In order to obtain a more rigid test of the resistance of this plant to serums, the fungus was planted in a soft medium consisting of agar one-half and the strongly agglutinating serum one-half. The mycelium grew well, and after one or two days' growth was inoculated with cholera vibrios. Within five hours the inoculated filament was swarming with actively motile vibrios, and the next day the filament and its branches were dead with vibrios penetrating to the finer endings. A portion of the medium removed with a capillary pipette and applied to a hanging drop of cholera vibrios caused immediate clumping. It is evident from this experiment that infection with high motility of vibrios goes on unchecked, even when the host is grown in a highly agglutinating medium.

To summarize, the cellulose wall of the fungus is apparently an effective barrier against the agglutinins of both cholera and *Bacillus pyocyaneus* serums.

Somewhat different results were obtained as regards the permeability of this plant for acids. Since *Achlya* will tolerate a degree of acidity in the medium decidedly harmful to cholera vibrios, it was possible to determine if the progress of an infection might not be altered by changes in the reaction of the medium.

As a protocol a detailed description of one of this series of experiments is given:

April 4, morning. A small mycelium grown from a resting spore was placed in a hanging drop of the water of condensation of alkaline agar to which a small quantity of nearly neutral glucose broth had been added.

April 4, 5.30 p. m. Above medium removed and cholera peptone plus about 0.1 volume of glucose broth substituted.

April 5, 10.15 a. m. (room temperature 28°.6). Inoculated with a fresh culture of cholera vibrios.

April 5, 11.00 p. m. Filament infected, with motile vibrios present. Withdrew alkaline medium and substituted an acid medium consisting of distilled water 5 parts and 1 per cent glucose broth, 5 + acid, 3 parts. A previous test had shown that cholera vibrios were unable to grow in a hanging drop of this medium.

April 6, 9.30 a. m. (temperature 28°.3). A portion of the inoculated filament is dead, but with few vibrios in it. Branches 1 and 2 are living and contain vibrios with little or no motility.

Actively motile vibrios added to the hanging drop immediately clumped and became motionless.

April 6, 12.10 p. m. Withdrew medium and added fresh of the same kind. This was repeated at 7 p. m. the same day.

April 7, 8.30 a. m. Branch 1 still living and healthy with thick protoplasm. The length of the branch has nearly doubled since the second day of infection. Few vibrios are present inside of filament, none of them motile.

In order to determine if the vibrios could be revived, the medium was withdrawn and one substituted which contained but 1 part of the acid glucose broth in 8 of water. A previous test had shown that vibrios multiplied freely in this fluid with active motility.

April 7, 4.45 p. m. The protoplasm in branch 1 is apparently more actively motile. Few vibrios within it and these tending to clump. None outside in that part of the culture.

April 8, 8 a. m. (temperature 29°.8). A segment in the middle of branch 1, including about 0.4 of the whole length, is still living with protoplasm actively motile. Withdrew medium and added fresh of the same kind.

April 8, 7 p. m. The living portions of the filament are growing into the dead portions. No vibrios found inside on examination with $\frac{1}{2}$ oil immersion lens.

April 9, 7 a. m. Portion of branch still alive.

April 9, 3.30 p. m. Branch apparently dead. No vibrios found inside, but are multiplying in the outside medium.

Summary.—An infection of this filament took place, but was checked apparently in response to treatment with an acid broth. The filament lived four days with conditions under which non-treated infected filaments have usually died within twenty-four hours. The final death of the filament—like that of the controls—was evidently due to causes other than the cholera infection.

In another experiment a plainly infected filament was apparently "cured" in the same way. In other experiments infection had gone so far that the vacuole of the filament was well filled with actively motile vibrios. Here infection could be checked, and the vibrios made to clump by acid treatment; but in those filaments which survived treatment the vibrios sometimes became active again. In some cases acid-treated vibrios inside living filaments took on monstrous forms, some becoming thick spirals and some amoeboid in form while still retaining their motility. In one infected filament the protoplasm of the

host massed into a sphere containing no or very few vibrios, took on a wall, and later grew into the infected part, now dead. It was found more difficult to infect filaments in even a slightly acid medium.

In summary, *Achlya* filaments in which cholera infection is not too much advanced may apparently be "cured" by the addition of a weakly acid medium, but the wall and protoplasm of the living plant evidently offers some resistance to the passage of the acid. Vibrios inside were less affected than those without, and highly infected filaments were difficult to cure.

The dysentery bacillus inoculations were made with an old culture of the Shiga-Kruse type. Some four successful infections were accomplished. While the bacilli grew well, in some cases filling the filament for a considerable distance from the point of inoculation, the destruction of the host was much less rapid than in the case of the motile bacteria. In one experiment an infected filament lived nearly, if not quite, five days at high room temperature. During at least three days of this time the bacilli were numerous in the host; but its protoplasm continued to move, though in contact with dense masses of bacteria. In another case an *Achlya*, taken directly from a water culture among algæ, was inoculated. A portion of the infected mycelium lived six days after infection.

Bacilli of bubonic plague also grew well in the living filaments of *Achlya*. In one experiment a highly virulent culture, the first twenty-four hours' growth from an infected guinea pig, grew well, and formed chains which extended far from the point of inoculation and into neighboring branches. The hypha inoculated and other parts of the mycelium in connection with it died within twenty-four hours. After the death of the host rhizoid-like filaments from neighboring hyphæ surrounded and penetrated the infected filament apparently obtaining food from the disorganizing mass of bacilli. A similar attraction of masses of bacteria for *Achlya* was seen in dysentery infections. This behavior of *Achlya* does not prove that the bacteria possess no toxins for *Achlya*, since toxins, like agglutinins, may be unable to pass the cellulose wall. However, in dysentery at least, there is little evidence of the formation by bacteria inside the wall of toxins destructive to the host.

Some interesting results were obtained by the inoculation of spores of an *Aspergillus* isolated from moldy bread. A single spore was inoculated into the vacuole of a filament of *Achlya* growing in pure culture in a hanging drop of glucose agar. In

twenty-four hours the spore had germinated and put forth a long hypha which grew rapidly within the host and formed septa.

On the second day after inoculation the *Aspergillus* mycelium had branched extensively, spreading through the infected hypha and far into its branches. The invading hyphæ tended to follow the wall of the host, lying in the layer of protoplasm. The *Achlya* was still living with motile protoplasm two days after inoculation, but the protoplasm was becoming scanty. In many places branches of the *Aspergillus* grew through the wall of the still living host and penetrated the surrounding medium. (Plate III.)

Here the *Aspergillus*, apparently an ordinary saprophyte, traverses the wall of the living host from within outward in the same way that a true parasite enters. The penetration of the wall is accomplished in both cases with little or no loss of contents or immediate disturbance of the protoplasmic circulation of the host. No case was observed of *Aspergillus* branches outside penetrating a new *Achlya* filament.

During the second day the *Achlya* filament died, filled with the mycelium of the *Aspergillus*. There were no bacteria in the neighborhood, and the death of the host was apparently due to exhaustion of food. One or two days later branches of the *Aspergillus* coming from the *Achlya* filament formed fruit stalks and spores.

In this experiment we have a saprophyte, or certainly a plant not normally parasitic on *Achlya*, behaving much like a parasite when its spores are mechanically introduced into the other plant.

Yeast plants also grew well in the hyphæ of *Achlya* growing in pure culture. Both fungus spores and yeast plants are comparatively difficult to inoculate, since one must use a pipette of relatively large size in order to admit these cells into it, and such a pipette must necessarily make a large opening in the wall of the filament inoculated.

SUMMARY

I. *Achlya* growing in pure culture is readily infected with *Bacillus pyocyaneus*, cholera vibrios, or the bacilli of dysentery and of bubonic plague, when inoculated into the cell cavity.

II. *Pyocyaneus* or cholera-infected filaments usually die within twenty-four hours after inoculation. Filaments infected with dysentery bacilli sometimes survive five or six days at a room temperature of from 27° to 31°.

III. Yeast plants and spores of *Aspergillus* also develop in

Achlya, the latter behaving much like a true parasite when inoculated under these conditions.

IV. Parasitism rather than intoxication apparently plays the larger part in these infections.

V. Aside from the occasional walling off of a less infected portion, *Achlya* shows little power of protecting itself against an infection introduced within the filament.

VI. An infection with cholera may be delayed, or, when not too far advanced, entirely arrested by the application of weakly acid media outside the filaments.

VII. Agglutinins of both cholera and *Bacillus pyocyaneus* in specific serums apparently fail to penetrate in the slightest degree the cellulose wall of the fungus.

ILLUSTRATIONS

- PLATE I. A mycelium of *Achlya* infected with *Bacillus pyocyaneus*. The infected filaments show dark in the photograph because packed with bacilli which took the stain (carbol fuchsin) deeply. The dark rounded masses are composed of bacteria forced out during the process of growth.
- II. A mycelium of *Achlya* infected with vibrios of Asiatic cholera. The dark-staining filaments are packed with bacteria, and a few extruded masses of bacteria are shown.
- III. A filament of *Achlya* infected with *Aspergillus*. The outgrowth of filaments of the *Aspergillus* through the walls of the host is shown at the end and the side of the *Achlya* filament.



PLATE I. A MYCELIUM OF ACHLYA INFECTED WITH BACILLUS PYOCYANEUS.

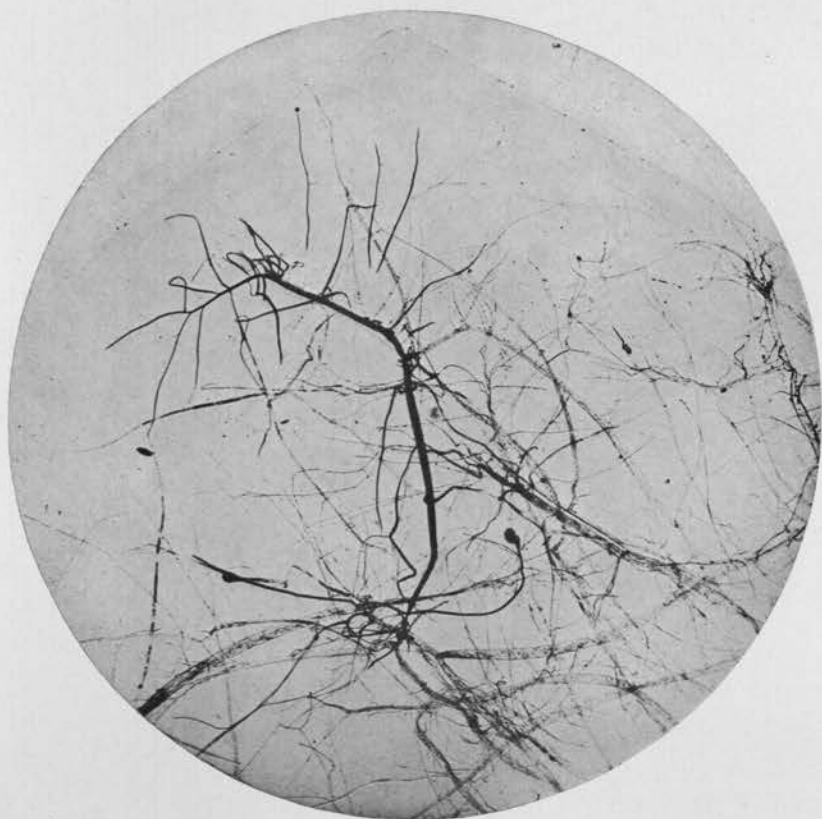


PLATE II. A MYCELIUM OF ACHLYA INFECTED WITH VIBRIOS OF ASIATIC CHOLERA.

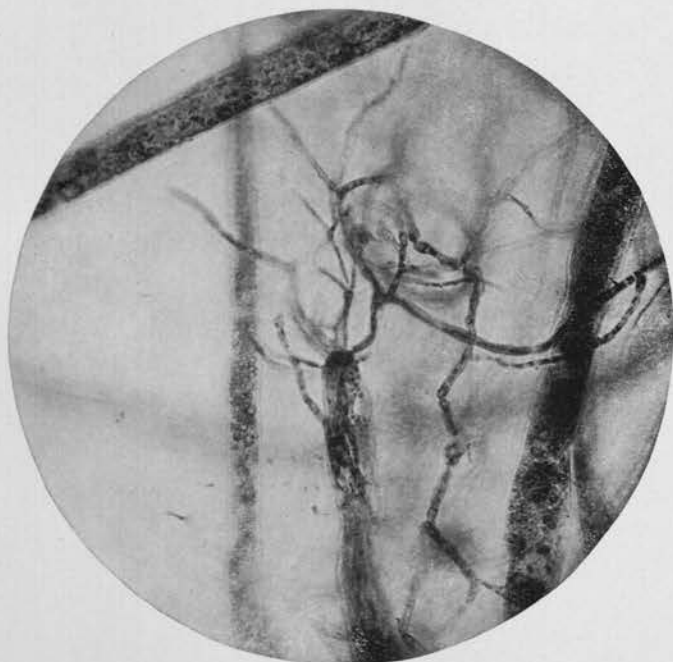


PLATE III. A FILAMENT OF ACHLYA INFECTED WITH
ASPERGILLUS.

ACUTE MALIGNANT GLANDERS IN MAN

By W. E. MUSGRAVE and A. G. SISON

(From the Clinics of the College of Medicine and Surgery, University of the Philippines, and the Philippine General Hospital, Manila, P. I.)

One plate

There is a form of acute malignant rapidly fatal glanders in man, the importance of which is not sufficiently emphasized in the current literature of the subject.

The four appended condensed case records form the basis for the present discussion of this subject.

INCIDENCE

Human glanders, except in certain parts of Russia, is a rare enough disease to make individual case reports of interest because the diagnosis often, if not usually, is not made early in the disease. For example, Meyer and Crohn¹ state that only 9 cases were reported in the city of New York during two years. When one considers the very contagious character of this infection, together with the very close association between human beings and horses, particularly in the Philippine Islands and most other tropical countries, it is surprising that human glanders is not more frequently encountered. The four cases from Manila would suggest a greater incidence than is generally recognized in this country.

CASE I. ACUTE MALIGNANT GLANDERS—DURATION OF ILLNESS FOURTEEN DAYS—DEATH

Patient 599.—Filipino, male, 18 years of age, coachman by occupation. Admitted to the hospital complaining of very high fever, severe pains in the joints, and headache. Family history and history of previous diseases unimportant.

Present illness.—Eight days before admission, patient was taken suddenly ill with fever, headache, and nausea with occasional vomiting. Within twenty-four hours, joint pains developed, starting in the right knee and spreading rapidly to nearly all of the important joints of the body. These pains were severe and acute in character, and a considerable amount of œdema in and about the joints developed very rapidly. No external wound nor primary lesions of the mucous membrane were discovered.

On admission to the hospital on the eighth day of his illness, the patient was very ill; he had high fever and a rapid pulse, a general toxic appearance, and his joints were swollen, painful, and tender to the touch. On this date a single superficial pustule about 1 centimeter in diameter was noticed on the left side of the neck, and within forty-eight hours a considerable number of these lesions were found scattered over the body, particularly on the face and chest. The lesion was a particularly typical one (Plate I). It appeared suddenly and developed rapidly, starting as a vesicle, but within a few hours became pustular. It was superficial in character, umbilicated, and surrounded by a marked inflammatory zone. In appearance these lesions very closely resembled ecthyma. They varied in size from 5 millimeters to 1 centimeter in diameter, were easily broken, and *Bacillus mallei* was cultivated from the contents practically in pure culture.

The only important clinical laboratory findings in this case was the blood. The leucocytes, on the day of admission, were 7,800 with

	Per cent.
Lymphocytes	11
Large mononuclear	5
Polynuclear	81
Eosinophiles	1
Transitional	2

Three days after admission and when the eruption was fully established, there were 9,000 leucocytes with

	Per cent.
Lymphocytes	7
Large mononuclear	13
Polynuclear	77
Transitional	3

On the twelfth day of the disease, the patient's general condition was much worse. The vesicular eruption had extended to the face and trunk. Some of the vesicles were umbilicated, a few were pustular. A clinical diagnosis of glanders was made by one of us (Musgrave), and the diagnosis was confirmed by the usual laboratory methods two days later.

The patient suffered intensely with joint pains during the two following days. The fever ranged from 38° to 39°.5 C.; there were frequent irregular chills and great prostration and toxæmia. Death occurred on the fourteenth day of the disease.

AUTOPSY REPORT

Anatomic diagnosis.—Papulo-pustular eruption of skin; glanders; multiple small abscesses of pectoral muscles; large abscesses near ankles; pyarthrosis; pyæmia; beginning abscesses and posterior congestion and œdema of lungs; subserous ecchymosis of heart; fatty and albuminous degeneration of kidneys; acute exacerbation of splenic tumor.

Pathologic diagnosis.—Body of fairly well-nourished Filipino; rigor mortis moderate; skin of face, chest, abdomen, and extremities show numerous, but not conglomerate, papulo-pustular eruptions, averaging 1 centimeter in diameter, raised about 2.5 to 3 millimeters above the surface. They are dull gray in color, and very few are umbilicated; all contain a gray mushy pus, emitting an odor suggestive of mice stools. Stray ulcers

are seen where a papulo-pustule has ruptured; the edges of these ulcers are slightly beveled, neither punched out nor undermined; their bases are covered with gelatinous sloughs. The palms and soles are free; the post-cervical lymphatic glands are numerous and faintly palpable. There is no scar on the penis; the mucous membrane appears to be normal.

The *subcutaneous fat* is moderate in amount and dry. The recti muscles are dark red; the pectoral muscles have numerous abscesses containing a gelatinous pus, measuring 1 centimeter deep and 5 centimeters wide. On dissecting away the skin, many of the pustules can be seen on the subcutaneous tissues, some having penetrated them. Large abscesses 4 by 3 by 2 centimeters are observed beneath the skin on legs, arms, and thighs. The joints of knees and ankles contain the same gray gelatinous pus, which in some places is fluid in character.

The *peritoneum* is gray, dry, and lusterless.

The *pericardium* contains a few cubic centimeters of clear, dull yellow serum. The heart shows numerous punctate and conglomerate hæmorrhages near the auriculoventricular septum. The heart is large and flabby; it was kept for a museum specimen.

Lungs.—Both pleural cavities are dry; there are a few recent firm adhesions between the right apex and upper part of chest wall. Both lungs are heavy, boggy, and œdematous; they do not crepitate well. They are blue-gray anteriorly and darker posteriorly. There are numerous dull blue-black areas 5 centimeters in diameter scattered over their surface. Cut sections ooze dark blood and froth, being blue-gray in front and darker in the posterior two-thirds. Anterior mediastinal glands are enlarged and œdematous.

Kidneys.—Fatty capsule preserved, foetal lobulations becoming obliterated. True capsules strip readily, leaving a dull yellow-red surface. Cut section is rather dry. The glomeruli and striations are invisible on the dull pale brown surface. Pyramids have lost their blue color and striations; same color as cortex. Suprarenals and ureter appear normal.

Liver.—Anterior border presents a blunt angle; color light brown, mottled with few large (3 by 4 centimeters) areas of dull yellow hue. Cut surfaces show a dull yellow color, oozing little blood, with lobulations obliterated. The dull yellow surfaces extend about 3 centimeters beneath the surface. Gall bladder is slightly distended with thick black bile.

The *pancreas* is dull pink and firm.

The *stomach* and *intestines* are moderately distended with gas. The mesenteric glands are large—more than 1 centimeter long—pale, dull pink, and firm.

CASE II. ACUTE MALIGNANT GLANDERS WITH SUPPURATIVE ARTHRITIS—DURATION OF ILLNESS FOURTEEN DAYS—DEATH

Filipino coachman, twenty-five years of age, previously in good health. Family and personal history negative.

Present illness.—He was taken ill rather suddenly with chills and fever and aching pains over the body. Irregular temperature and chilly sensations continued for six days. Arthritic pains developed on the fourth day, first in the right wrist, and spread rapidly to other large joints.

Patient came into our service on the seventh day of the disease with diagnosis of malaria and acute rheumatism. Examinations on the day of admission showed two papulo-pustular lesions, one on the right shoulder

and one on the right side of the neck. The temperature was 39° C. There was considerable swelling of a number of the large joints which also were very painful and tender to the touch. Patient was already obviously in a septic condition, and, although no external lesions of any description nor ulceration of the mucous membrane could be found, the diagnosis of acute malignant glanders was made from the appearance of the above-mentioned pustules. This diagnosis was confirmed by obtaining *Bacillus mallei* in pure culture from the pustule.

The laboratory findings in this case were as follows.

The urine contained a small amount of albumin, and occasional hyaline and granular casts were found.

The leucocytes were counted on the day of admission and again four days later. The first count showed 7,000 and the second 8,500 with very little disturbance in the differential findings.

Culture from the left knee joint obtained by aspiration gave a pure culture of *Bacillus mallei*. The patient grew rapidly worse from the day of admission, and died on the fifteenth day of the disease.

Incomplete *autopsy* showed a general suppurative arthritis, fairly numerous rather superficial skin pustules similar to that described in the first part, with a few small abscesses in the lungs and a few in the kidneys.

ADDITIONAL CASES OF ACUTE MALIGNANT GLANDERS IN MANILA

Wherry² reported 2 cases of this type of glanders. The following is abstracted from his report.

CASE I (WHERRY)

History (by Maj. Bannister, U. S. Army).—H. M., an American negro, 27 years of age, teamster, was admitted to the First Reserve Hospital on May 29, 1904, with a diagnosis of articular rheumatism. The patient had been ill for two weeks with chills, fever, and rheumatic pains.

While at the First Reserve Hospital he had a remittent temperature varying between 101° and 105° F., with an irregular morning rise and evening fall. A small pus sac was found posterior to the olecranon of the left arm. Aspiration yielded a few drops of lemon-colored serous liquid which, microscopically, contained numerous pus cells and a few capsulated rod-like bacteria. Later, a larger pus sac was found on the dorsum of the left foot.

Sputum examination.—May 30, mucopurulent, containing a few blood cells; no tubercle bacilli. *Blood examination*, May 29, 21,000 leucocytes; June 3, 23,400 leucocytes; no parasites. *Urinalysis*, May 29, 1,021; alkaline, trace of albumin; no casts, many leucocytes. June 2, 1,019; alkaline, no albumin; no casts, few leucocytes; bile.

Diagnosis.—Pyæmia; acute articular rheumatism, both elbows, knees, and ankles; suppurative inflammation of left elbow (type undetermined).

About June 6, he developed a cutaneous eruption which was considered to be that of smallpox, since a smallpox patient had been removed from an adjoining bed seven days before the eruption appeared on H. M. He was removed to the military smallpox hospital, and died on the following morning. The body was sent to the city morgue marked "suspected smallpox case."

² Bureau of Government Laboratories, Manila (1904), No. 24.

Incomplete autopsy 387 (about twenty-four hours after death).—No autopsy was requested, but Dr. W. E. Musgrave and Dr. W. R. Brinckerhoff, who happened to be at the morgue, noticed the peculiar cutaneous eruption and brought pieces of the skin to the laboratory for further examination. Large areas of skin surface were covered with the numerous, closely set vesicles of miliaria, and among these were numerous pustules of varying size. Some of these pustules were capped by a vesicle, which in some instances showed a central depression giving an appearance of umbilication.

None of us thought of human glanders until a microscopic examination showed that pustules contained numerous bacteria morphologically resembling *Bacterium mallei*—rods about the length of, and somewhat thicker than, the tubercle bacillus, which stained irregularly with Löffler's methylene blue and lost the stain in Gram's method. No such bacteria were found in smears made from the abscess in the left ankle.

Bacterium mallei was isolated by the usual bacteriologic methods.

Histologic examination (by Doctor Brinckerhoff).—A section through one of the larger pustules (about 4 millimeters in diameter) shows a densely infiltrated area in the skin and subcutaneous tissues. This inflammatory exudate lies chiefly between the muscular layer and the Malpighian cells of the epidermis. The epidermis is raised to a considerable distance above its level in the adjacent normal skin. At the point where it leaves its normal level the deeply pigmented cells of the rete malpighii are seen to be greatly elongated, and just before it reaches its greatest elevation there is a splitting away of the horny layer, which, continuing to a similar point on the opposite side, leaves a space which constitutes the vesicular portion of the pustule.

Under a high-power lens the contents of the vesicle is seen to be composed of degenerated polynuclear leucocytes and cells from the stratum granulosum, nuclear fragments, and a granular detritus which represents the products of cell degeneration and coagulated serum. Beneath the area the cells of the rete show marked infiltration and vacuolation, many of their nuclei staining but faintly or not at all. The deeper infiltrated area is composed of a dense collection of more or less degenerated leucocytes and erythrocytes, degenerated epithelial cells, and nuclear fragments, many of which seem to have coalesced to form irregular, deeply staining masses of chromatin. Here some islands of degenerating epithelial tissue, probably the remains of papillary pegs, may be seen. No giant cells are present. A very prominent feature is the widespread and extensive destruction of the nuclei of the fixed and infiltrating cells. This varies from simple vacuolation to complete karyorrhexis. A number of normal polynuclear cells may be seen with their protoplasm filled with rounded or rod-shaped nuclear fragments. The blood vessels of the subcutis show great congestion. The underlying muscular and glandular tissues appear normal. There are no signs of proliferation—everywhere those of degeneration.

CASE II (WHERRY)

A. C., Filipino, 38 years of age, married, a clerk by occupation, was taken sick on October 2, 1903, with chills and fever. In December of the same year he had an abscess on the posterior portion of the right leg, which was opened by the physician who attended him. Again, in February, 1904, he had an abscess in the anterior and upper portion of the right side of the chest, which underwent resolution without operation. On June 27, 1904,

an eruption appeared on the face and later on the chest and abdomen. On the third day of the eruption the patient died. The patient was the owner of a stable where horses and vehicles were kept for hire; he had recently lost several horses from glanders.

Autopsy.—The body appears emaciated. A papulo-vesicular eruption is scattered over the skin surface. These lesions are most numerous on the face, back of the trunk and upper arms, buttocks and back of the upper part of the thighs, and more scattered over the chest and the abdomen. They are not distributed regularly, but are grouped with intervening areas of comparatively free skin, and vary in size from 2 to 3 and 6 to 8 millimeters in diameter. The smaller ones appear as shotty papules, while the larger ones are distinctly vesicular. Several of these vesicles show depressed areas, which give the appearance of being umbilicated, although more commonly their surfaces appear wrinkled. On section they are seen to be situated on a fairly well-defined, yellowish, firm nodular base, which extends into the subcutaneous tissue. The eruption on the face seems to be more advanced. On the forehead and cheeks the lesions appear as irregularly circular, raised, dark red and purple-colored plaques, about 1 to 1.5 centimeters in diameter. Some of these are rounded and nodular, others flat with depressed center and raised edges. A few present a reddish yellow, ulcerated center surrounded by raised edges. Two of these nodular plaques occur beneath the skin of the scalp, just above the upper margin of the forehead.

The tissues just to the right of the nose are so swollen as to close the right eye, which itself does not seem to be affected. A section through these swollen tissues reveals many discrete and confluent, yellowish and grayish, caseous foci, which are surrounded by congested and necrotic tissue and extend to the depth of about 2.5 centimeters from the skin surface. The *alæ nasi* are thickened by similar nodules, and one can be seen on the mucous membrane of the upper lip, just to the left of the nasal fossa. A dirty, purulent discharge escapes from the nostrils. Upon opening the nares, the mucous membrane, especially of the right one, is seen to be ulcerated. The ulceration covers most of the mucous surface of the floor and walls of this nostril, and extends upon the inferior turbinated bone. Small, grayish or yellowish nodules may be seen projecting from the ulcerated surfaces. The right nostril is not affected to such a marked degree. The ethmoidal cells are filled with purulent matter. The right clavicle is much thicker than the left, but nothing of note is seen on section.

The thoracic cavity contains no fluid. Both lungs are bound to the thoracic walls by firm, fibrous strings of adhesions. The lungs are emphysematous anteriorly, and posteriorly show considerable hypostatic congestion. On palpation small nodules can be felt beneath the visceral pleura, which on section appear as pea-sized or smaller, circumscribed, grayish yellow areas of a confluent tubercular structure. None of them are capsulated or caseated, but some are surrounded by an irregular, reddish area of pneumonic consolidation. They seem to be limited to the pleural surface.

The bronchial glands appear normal on section. The trachea and bronchi are slightly congested, and are covered by a mucopurulent secretion. The heart muscle is rather pale on section, but otherwise the organ appears normal. The mucous membrane of the *œsophagus* shows hyperplasia of the solitary follicles.

The liver is of about normal size, soft, and on section its markings are

indistinct. The spleen is somewhat enlarged, soft, and on section its pulp is diffuent. The kidneys are slightly enlarged, their capsules strip readily, and on section the cut surface is yellowish white and the cortical and medullary markings are very indistinct. The stomach and intestines were not opened. No further examination was made.

Bacteriologic examination (by Dr. W. R. Brinckerhoff).—The twenty-four-hour cultures on glycerin agar showed numerous barely visible, transparent colonies. In forty-eight hours these colonies became visible, and in pure cultures gave the biochemical reactions which have been described as characteristic of *Bacterium mallei*.

The *histologic* changes in the subcutis and lower layers of the cutis are similar to those described in case I. The process, however, is not so far advanced, for, although karyorrhexis is widespread and prominent, it is not so marked in the upper layers of the cutis, where the chief changes are a loss in the staining power of the nuclei and a general vacuolation of the cell protoplasm. The stratum corneum has not been split off and consequently the pustule is not covered by a vesicle.

Lung.—A section through one of the subpleural nodules (about 3 millimeters in diameter) shows, under a low magnification, an irregular area of consolidation characterized by intense infiltration of the pulmonary alveoli and marked congestion of the blood vessels of the alveolar walls and of the pleura covering the affected area.

Under a higher power the contents of the alveoli is seen to be composed chiefly of polynuclear and transitional leucocytes, a few lymphocytes, pigment-carrying cells, and a few large cells which lie, for the most part, near the alveolar walls and resemble desquamated endothelial cells. It is apparent that many of the cells in this area are undergoing degeneration and karyorrhexis, but not to so marked an extent as in the skin pustule of case I. This area is surrounded by pulmonary tissue which shows intense congestion and in which the alveoli are filled, for the most part, with extravasated blood, granules, and threads of fibrin, desquamated endothelial cells, and a few polynuclear and transitional leucocytes. Deeper within the section the alveoli appear normal.

In another field of the section several small foci of infiltration may be seen, situated at some distance beneath the pleural surface, and each is about the size of a single air cell. Their contents is composed of cells similar to those found in the larger focus, but karyorrhexis is not so marked a feature. One of these appears to have ruptured into an adjoining alveolus, and such a process may indicate the histogenesis of the larger foci. All the foci appear to be recent ones, and there are no signs of proliferation or encapsulation. No giant cells are to be seen.

DISCUSSION

Etiology.—The disease is caused by a general infection with *Bacillus mallei*, and consequently its spread must conform to the usual well-known methods of transmission of diseases caused by bacteria.

When this fact is considered together with the very great virulence of the organism both in human beings and in some of the lower animals, the comparative rarity of the infection in man

is difficult to explain, particularly in countries where the incidence of infection in animals is very high.

The habits and customs of coachmen in Manila, for example, and the close housing of man and horses and other animals, give the most favorable opportunities for the spread of the disease to man. If there is a natural immunity in man against the infection, this immunity certainly has a very irregular distribution and must be very strong in a large percentage of people, entirely absent in a few, and of moderate degree in practically none. Variation in virulence in the various strains of the bacillus does not answer the question because the disease practically always is fatal in horses, and, when man becomes infected, the disease usually is a particularly virulent one and cases of mild infection are almost unknown.

Pathologically the most striking peculiarity of *B. mallei* infection is the marked predilection of the organism for joints, lymphatics, and cutaneous tissue. In practically every instance there is a more or less extensive suppurative arthritis and suppurative lymphadenitis and a more or less extensive distribution of suppurative skin lesions.

Inflammatory areas and abscesses in other tissues and organs of the body frequently develop toward the end of the disease, but the principal and early and constant lesions are as above mentioned.

Symptoms.—The period of incubation is unknown. The onset is similar to that of a number of severe acute infections; usually, it is rather sudden with chill or chilly sensations with fever and indefinite aching pains. In some cases there is a prodromal period of indefinite symptoms and a more gradual development of fever. In still other cases the onset resembles that of pneumonia very closely, and it may not be possible positively to differentiate the two conditions until the absence of the expected consolidation is noted.

The fever usually runs a more or less irregularly remittent course, varying from 38° to 40° or even 41° at times.

The pain at first may be more or less general in character, similar to that usually seen in dengue. More often, however, from the first and in all cases after the disease is fully established the pains localize in the joints—particularly the larger joints—as the elbows, knees, ankles, etc.

The joints rapidly become swollen and painful and the skin glistening, as is seen in rheumatic fever. Still later, fluid accumulates, and in the course of a few days suppuration is found

in one or more joints. The aspirated contents of the joints shows pure culture of *B. mallei*.

The lymphatic glands in various parts of the body and particularly around the primary focus of infection, when one is present, rapidly become swollen and tender and gradually suppurate, and in some instances, when the patient lives long enough, break down and cause open ulcers. In cases I and II that are reported here, there was, however, striking absence of enlargement of the lymphatic glands that could be detected by physical examination, and there was but slight lymphadenitis. Exception is made of the mediastinal glands which were found at autopsy slightly enlarged and œdematous.

The skin lesion is a very striking and characteristic one. The lesions never are exceedingly numerous, and sometimes only a few will be found until toward the end when they usually become very numerous. They appear one or a few at a time, and, while showing a predilection for the face, neck, back, and chest, may be seen on any part of the body. The first skin lesions usually appear in from four to seven days after the onset of the disease, and others continue to appear throughout the course of the disease. However, we have seen one patient who did not have more than 20 of these lesions before the twelfth day of the disease.

The lesions appear at first as simple superficial papules which rapidly enlarge and soon become vesicles, then pustules, and then break down and become open sores. The lesions always appear to be superficial, although careful examination reveals a surrounding area of infiltration, and section shows that the infiltration extends through all layers of the skin.

During the vesicular stage, the superficial raised character of the lesion resembles that seen in impetigo contagiosa. Cultures from the skin lesions usually show pure culture of *B. mallei*.

One very striking clinical feature of acute glanders is the marked depression and general appearance of serious illness that develops early in the disease and continues throughout its course. The patients gradually sink into unconsciousness a few days before the end.

Diagnosis.—The disease frequently is not diagnosed during life and rarely during its early stages. This, too, in spite of the fact that the diagnosis is very easy to make clinically and its confirmation by laboratory methods a simple and easy procedure.

The reason why the diagnosis is not more often made is due to the rarity of the infection. Most physicians never have seen

a case, and it, therefore, is not included in the routine mental pictures that one examines in connection with cases of fever, rheumatic pains, and skin lesions.

Glanders is most frequently mistaken for dengue fever, rheumatic fever, syphilis, or typhoid fever, and less frequently for pleurisy, pneumonia, and certain skin diseases, particularly impetigo contagiosa, pemphigus, or ecthyma.

During the early stages before the appearance of the characteristic skin lesion, the clinical picture of glanders may resemble dengue or rheumatic fever very closely, but careful examination will justify distinction even at this stage. Dengue may be eliminated positively and absolutely by the well-known blood picture of this disease.

The mode of onset, the character of the pains, the fever, and the blood picture of glanders and rheumatic fever may be indistinguishable, and, as it often happens in acute glanders cases that there is no visible primary lesion, the diagnosis may remain in doubt until suppuration in the joints or the skin lesions make the diagnosis clear. The value of early blood cultures as a diagnostic method in glanders needs further study.

The only excuse for confusing glanders with the skin diseases mentioned is in the similarity of the local lesions. The pronounced constitutional manifestations of glanders should obviate this mistake more often than it does.

The *prognosis* in this form of glanders is bad. The disease probably is a general infection practically from the beginning and rapidly becomes a virulent pyæmia.

There is no known treatment that has any curative properties or that influences the course of the disease further than to alleviate some of the distressing symptoms that supervene before unconsciousness develops.

ILLUSTRATION

PLATE I. Appearance of skin in acute malignant glanders in man.

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PLATE I. APPEARANCE OF SKIN IN ACUTE MALIGNANT GLANDERS IN MAN.

NOMA IN THE PHILIPPINE ISLANDS WITH REPORT OF A CASE ENDING IN RECOVERY

BY C. M. REYES

(From the Clinics of the College of Medicine and Surgery, University of the Philippines, and the Philippine General Hospital, Manila, P. I.)

One plate

The problems of epidemic diseases and intestinal parasitic infections have received a great deal of attention from writers on tropical conditions, but literature on the more common clinical diseases is not so voluminous, and very little mention of the incidence of noma in hot countries is to be found. Yet it is a fact that noma does exist and makes its ravages among children in tropical climates; it is spoken of as being fairly common in Korea and Formosa, and it is occasionally met with in China. In India it is said to be especially frequent in adults.

It is a noteworthy fact that in the Philippines, at least, measles, which is the one eruptive fever most commonly followed by noma, rarely ever assumes the gravity it does in the more temperate zones, like the United States, for example. Whether this may account for the apparent rarity of the malady in this and other tropical countries, as judged by the scanty literature on the subject, is open to question.

The several cases of noma seen at the Philippine General Hospital were unfortunately seen too late in the course of the disease to justify surgical intervention. All such patients have succumbed to the effects of a most virulent sapræmia. In the following case the disease developed while the patient was being treated for empyæma in the hospital, and is reported on account of the extreme rarity of recoveries from this fatal affection.

REPORT OF CASE

Patient.—A. O., 2½ years of age, male, born of Filipino parents.

History.—Patient was admitted to the hospital May 17, 1912, complaining of fever and cough of one month's duration. After examination, a diagnosis of empyæma on the left side was made and drainage instituted. The temperature gradually subsided, and the patient did well up to August, when he began to develop

a high, irregular temperature and was steadily failing, until by the latter half of September it was deemed necessary to submit him to a second operation by enlarging the old drainage wound. Condition of the patient remained but little changed.

On October 1, 1912, the patient's upper lip was noticed to be swollen and oedematous and further examination showed slight necrosis of the mucosa opposite the insertion of the teeth. The necrotic tissue was scraped, and the exposed surface cauterized with pure phenol. By the next day the necrotic process had extended to the inside mucosa of the lower lip and a small part of the gums of both upper and lower jaws. Four front teeth were removed because they were found to be so loose that there was danger of their being swallowed. The same treatment as on the previous day was instituted, plus frequent swabbing of the parts with hydrogen peroxide, and later with a potassium permanganate solution.

On October 3, the skin over the upper lip became glossy, and by the fifth day the gangrenous process had invaded new tissues outward, so as to present a typical picture of *cancrum oris*—a foul, ashy gray, pultaceous mass, involving the entire upper lip (Plate I, fig. 1).

It was impossible for the patient to take anything but liquids, and his low state of health from the original trouble did not warrant a more active surgical interference, which would have necessitated general anaesthesia. The treatment outlined above was continued except that iodine was substituted for phenol on account of the exposed situation of the parts. The course of the lesion was progressive, until by the tenth day the floor of the nose also was involved, and the patient presented a pitiful appearance. The odor from the sloughing necrotic tissue was most offensive.

There was no further extension of the process, a line of demarcation gradually formed, and by the twentieth day of the disease the margins of the ulcer began to show a more healthy appearance, and the general condition of the patient showed slight improvement. The favorable local changes continued until healing was complete, but the process had destroyed so much of the upper lip that the middle of the upper jaw was left exposed. The patient has not yet sufficiently recovered from his original affection to justify a plastic operation (Plate I, fig. 2).

Examination of smears from the slough showed long spirilla

and spindle-shaped organisms, and blood culture gave *Staphylococcus aureus* and *albus*.

Noma is essentially a disease of children, especially under 10 years of age, but it has been met with in adults. Girls are more often affected than boys.

It usually follows or develops during the course of some debilitating sickness, like the eruptive fevers, particularly measles and typhoid. It has been met with after scarlet fever, whooping cough, bronchopneumonia, diphtheria, variola, etc. Unhygienic surroundings, underfeeding, and cachexia are strong predisposing causes, but it has been met with where the sanitary, hygienic, and dietetic conditions were most favorable. Usually sporadic in its appearance, the disease has been observed from time to time in epidemic forms, which, together with the fact that it seems to have a special predilection for the lining mucous membrane of the different orifices of the body, strengthens the belief that it is of a specific infectious nature. Formerly the malady was considered to be the result of some vasomotor or trophic disturbance, but in the light of the present bacteriologic researches it must be considered as an infectious disease due to a definite cause, and the isolation of the specific microorganism may be predicted with confidence.

Blumer and MacFarland, after studying a series of 16 cases, arrived at the conclusion that the disease begins primarily as a simple infection and terminates as a mixed infection, generally with a slim organism of the leptothrix type predominating. Weaver and Tunnicliffe, who have compiled the results of the bacteriologic studies of noma by several investigators, report the almost constant finding of polymicrobial cultures from the superficial areas of necrotic tissue, while the deeper portions showed long, thread-, or filament-like bacteria in almost pure culture, the bacteria being easily decolorized by Gram, but not growing on ordinary culture media.

The disease usually attacks the mouth and cheek, but may occasionally attack the vulva and anus (noma pudendi). Perhaps this may explain, in part, its greater frequency among girls.

It usually begins as a livid, swollen patch on the mucous membrane of one of the cheeks, followed by an inflammatory infiltration which is rapidly transformed into typical gangrenous tissue. The lesion soon spreads from the cheek, which becomes a foul, ashy gray, pultaceous slough, and the process often extends

to the adjacent soft parts and underlying bones, so that in the very rare instance of recovery a subsequent plastic operation becomes necessary.

The prognosis is bad, about 80 per cent of the cases succumbing within ten days. Much depends upon the promptness with which treatment is instituted. Neglected cases invariably are fatal.

REFERENCES

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WEAVER and TUNNICLIFFE. *Journ. Infect. Dis.* (1907), 4, 8.
KEEN. *Surgery*. W. B. Saunders Company, Philadelphia and London (1910), 1, 344.

ILLUSTRATION

(Photographs by Cortes)

PLATE I

FIG. 1. Case of noma before recovery.

2. Same as fig. 1, after recovery.

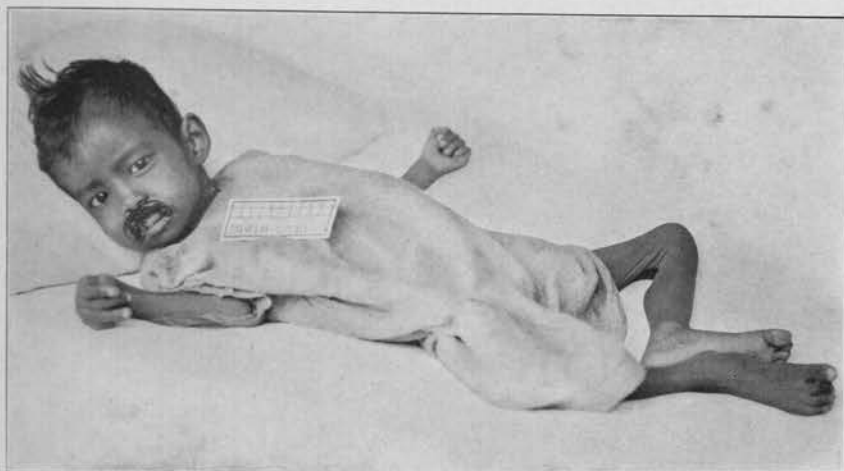


Fig. 1. Noma before recovery.

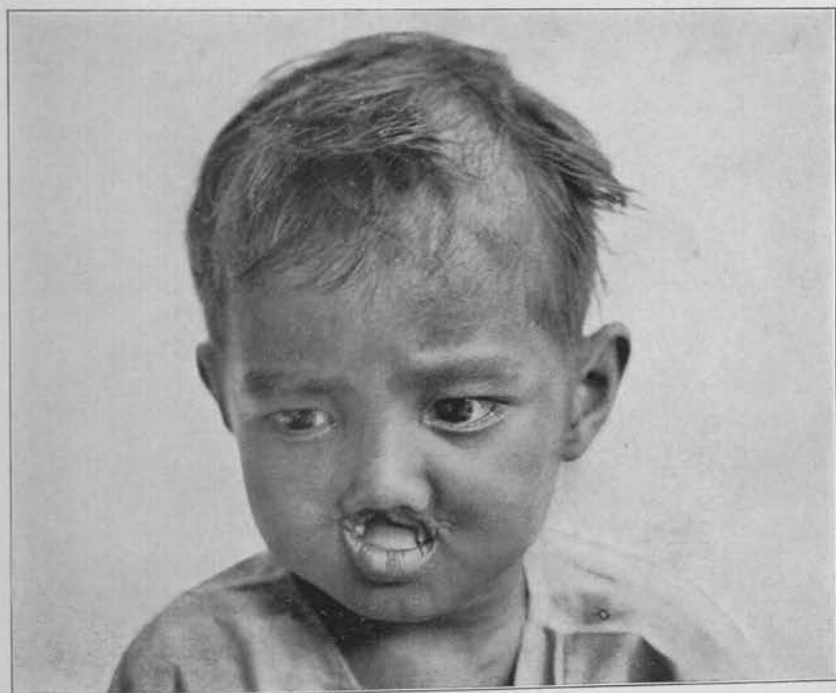


Fig. 2. Noma after recovery.

PLATE I.

CONCERNING VARIOLOID IN MANILA

BY P. M. ASHBURN, E. B. VEDDER, and E. R. GENTRY¹

(The United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

Both the Director of Health and the physician in charge of San Lazaro Hospital have several times spoken of the disease resembling varioloid that appears in Manila with a certain amount of regularity during each hot season and have expressed their uncertainty as to its nature. As a precautionary public health measure the cases have been classified as modified smallpox and isolated and treated as such. Having been particularly anxious to obtain access to cases of smallpox for the purpose of making experimental inoculations in monkeys, it has been our fortune to see a number of cases, all of which have shown great similarity and which are characterized in general by very slight, or no, fever and constitutional disturbance and by the appearance on the face, scalp, trunk, and limbs of a vesicular eruption, the lesions of which vary from 1 to 5 millimeters in diameter, are at times unilocular, at times multilocular, that very rarely umbilicate or pustulate, and usually dry to form brown scabs by the third or fourth day. The scabs fall off, leaving a small, pale mark without pitting. The lesions when first seen appear as mere papules roughening the skin, without redness. On the second day they are apt to be clear vesicles surrounded by a small area of redness. The lesions are most common on the face; next on the shoulders and front and back of the trunk, where they are distributed about equally; they occur less commonly on the arms and legs, and least so on the palms, soles, and scalp. In the cases seen the lesions were always discrete. A few red spots not to be positively identified with the skin lesions have been seen on the palatal surfaces; a few patients have spoken of mild sore throat. No complications or sequelæ have been observed. Some of the vesicles are elliptical in outline rather than round. The ages of the patients seen by us have varied from 12 months to middle life. The records show that there have been lately about 500 cases of "varioloid" in Manila per year, and Dr. A. P. Goff, physician in charge of San Lazaro Hospital, who has seen and treated the cases, states that most

¹ P. M. Ashburn, major; E. B. Vedder, captain; and E. R. Gentry, lieutenant; Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

of them are covered by the above description, and that there has been no mortality due to the disease.

We are of the opinion that of the cases occurring between July, 1912, and January 25, 1913, only one was smallpox or varioloid; and that case undoubtedly contracted the infection in China, as the disease developed a week after his departure from Hongkong and two weeks after leaving Shanghai. The other cases we think were not smallpox because:

(1) The cases were uniformly mild, the lesions like those of chickenpox rather than smallpox; shotty induration, umbilication, and pustulation were of rare occurrence.

(2) Previous successful vaccination or previous smallpox did not influence the occurrence or severity of this disease.

(3) Absence of both previous vaccination and previous smallpox did not influence it.

(4) Attempts (7 in number) to infect monkeys by inoculation with fresh vesicle contents were uniformly unsuccessful. Had the disease been smallpox most of the monkeys should have shown successful inoculations.

As illustrating the absence of influence of both smallpox and vaccinia on the occurrence of this disease, the following table shows the facts in regard to 15 patients admitted from January 4 to January 29, 1913.

Case No.	Age.	Date of admission.	Previous successful vaccination.	Previous smallpox.
	Yrs.			
1	8	Jan. 4	Yes; 6 months ago	No.
2	19	9	Yes; since admission	Yes; 15 years ago.
3	20	16	Yes; 6 months ago	No.
4	16	17	No	No.
5	13	19	Yes; in 1911	No.
6	18	18	No	No.
7	25	20	Yes; 16 days ago	No; mother of case 1.
8	14	20	No	No.
9	13	20	Yes; 6 months ago	No.
10	7	20	Yes; 1 year ago	No.
11	5	20	No	No.
12	1	22	No; attempt 6 months ago ..	No.
13	17	22	Yes	No.
14	1	25	Yes; Jan. 18	No; daughter of case 6.
15	6	29	Yes; date unknown	No.

We are of the opinion that the disease in question is not smallpox or varioloid and that it is, in all probability, chickenpox; and that smallpox, except for an occasional imported case, is nonexistent in Manila. If our opinion be correct, the facts signify a notable achievement in preventive medicine, and it is unfortunate that the records do not show it forth.

REVIEWS

Epidemic | Cerebrospinal | Meningitis | by | Abraham Sophian, M. D. | formerly with New York Research Laboratory | Twenty-three illustrations | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. i-xv + 1-272. \$3.00.

The author's large laboratory and clinical experience has enabled him to place before the medical profession a very valuable book on this subject. Several typographical errors detract from the appearance of the book.

J. A. J.

Tuberculin | in | Diagnosis and Treatment | by | Francis Marion Pottenger, A. M., M. D., LL. D. | medical director of the Pottenger Sanatorium for diseases of the lungs and | throat, Monrovia, California | with thirty-five illustrations, | including one plate in colors | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. 1-243.

This monograph is written by one who is so evidently an optimist that at times he seems to permit his enthusiasm to over-balance his judgment. It is on the whole a very good résumé of the treatment of pulmonary tuberculosis by vaccine therapy. The author lays commendable stress on the fact that this method of treatment is not one which lends itself to haphazard administration. In Plate I, figure 1, the explanatory legend to be correct should read "left" instead of "right," as it is the left eye depicted.

J. A. J.

Pellagra | History, Distribution, Diagnosis, Prognosis, | Treatment, Etiology | by | Stewart R. Roberts, S. M., M. D. | associate professor of the principles and practice of medicine, Atlanta College | of Physicians and Surgeons, Atlanta, Georgia; physician to the Wesley | Memorial Hospital; formerly professor of biology in Emory College | with eighty-nine special engravings | and colored frontispiece | St. Louis | C. V. Mosby Company | 1912 | Cloth, pp. 272. \$2.50.

Since 1907 when Searcy reported an epidemic of pellagra among the inmates of the Mount Vernon Hospital for the Colored Insane in Alabama, many articles relative to various phases of the disease have appeared in American and European medical literature. A book reviewing all this work was greatly needed by the busy practitioner. Such a book is Doctor Roberts's, and the

work has been performed in a very thorough manner. The section upon History and Distribution which naturally includes synonymy is particularly well executed, a map of the world and one of the United States showing the geographical distribution of pellagra being given.

While discussing the subject in general, the volume deals with it more directly as found in the South Atlantic portion of the United States. Doctor Roberts has seen the disease in Italy, and hence writes at first hand of pellagrous conditions in that country. It is obvious also that he has been in close personal touch with many of the investigators who have contributed largely to our present knowledge of the disease.

Symptomatology should have been included in the title. This section together with that on diagnosis apparently embraces most of the author's personal observations. A complete bibliography of the important literature on the subject would have added to the value of the volume.

D. G. W.

Diagnostic Methods | Chemical, Bacteriological | and Microscopical | a textbook for students and practitioners | by | Ralph W. Webster, M. D., Ph. D. | assistant professor of pharmacological therapeutics and instructor in medicine in | Rush Medical College, University of Chicago; director | of Chicago Clinical Laboratory | Second edition, revised and enlarged | with 37 colored plates | and 164 other illustrations | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut Street | 1912 | Cloth, pp. i-xxxvi + 1-682. \$4.50.

This book is designed for the use of students and practitioners, and it is to be commended for its marked lucidity and brevity in discussing laboratory diagnostic methods accepted by laboratory workers up to the date of its publication. Naturally it contains little that cannot be found in other publications on the same subject. The illustrations by Katharine Hill are excellent.

The portions of the volume which deal with the parasites of man are poorly executed; they are incomplete, and well-known rules of zoölogical nomenclature have been disregarded.

D. G. W.

Chloride of Lime | in | Sanitation | By Albert H. Hooker | technical director | Hooker Electrochemical Company | New York | John Wiley & Sons | London: Chapman & Hall, Limited | 1913 | Cloth, pp. i-vi + 1-231.

The author in a clear and condensed manner presents a résumé of the results obtained by the use of chloride of lime as a general

disinfectant and as a sterilizing agent for water and sewage. Not the least point of value in this book is a large bibliography. The volume should be valuable to all public health officials.

J. A. J.

Laboratory Methods | with Special Reference to the Needs of | the General Practitioner | by B. G. R. Williams, M. D. | member of Illinois state medical society, American medical association, etc. | assisted by | E. G. C. Williams, M. D. | formerly pathologist of northern Michigan hospital for the insane, | Traverse City, Michigan | with an introduction by | Victor C. Vaughan, M. D., LL. D. | professor of hygiene and physiological chemistry and dean of the department | of medicine and surgery, University of Michigan, Ann Arbor, Michigan | Second edition | illustrated with forty-three engravings | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. 1-210. \$2.50.

This little manual is exactly what the authors claim for it in the preface to the first edition, namely, a laboratory guide for the general practitioner. The fact that a prominent laboratory worker writes the introduction to the present edition speaks well for the merits of the book. It is moderate in price and should be of service to those for whom intended.

J. A. J.

The Narcotic Drug Diseases | and | Allied Ailments | Pathology, Pathogenesis, and Treatment | by | Geo. E. Pettey, M. D., | Memphis, Tennessee | member, Memphis and Shelby County Medical society, etc. [7 lines] | Illustrated | Philadelphia | F. A. Davis Company, publishers | 1913 | Cloth, pp. i-viii + 1-156. \$5.00.

Golden Rule Series | Golden Rules | of | Diagnosis and Treatment of Diseases | aphorisms, observations, and precepts on the | method of examination and diagnosis of | diseases, with practical rules for | proper remedial procedure | by | Henry A. Cables, B. S., M. D., | professor of medicine etc. [4 lines] | second edition | revised and rewritten | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. 318. \$2.25.

Cardio-vascular Diseases | recent advances in their anatomy, physiology, pathology, diagnosis and treatment | by | Thomas E. Satterthwaite, A. B., M. D., LL. D., Sc. D. | [11 lines, membership in societies, etc.] | [motto] | Lemcke and Buechner | 32 West 27th Street New York City | [no date of publication.] "Copyrighted 1913." Cloth, pp. 1-166, 80 text figs.

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